

SPRING ENVIRONMENTAL STRESSES AND FRUIT SET OF NAVEL  
ORANGES, Citrus sinensis (L.) OSBECK

By

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Two studies were conducted concerning the moisture balance of the low-yielding navel orange. The first study determined the influence of intermittent misting and antitranspirant sprays on plant water balance and fruit set. The second was a comparison of the morphological characteristics of the leaf and fruit stomata of navel orange with those of 2 other seedless cultivars, 'Valencia' orange (C. sinensis [L.] Osbeck) and the satsuma mandarin (C. unshiu Marcovitch), and the relation of these characteristics to their water balance. Both misting and an antitranspirant spray applied shortly after bloom improved the percentage of fruit remaining and the water balance of the tree as indicated by increased leaf water potential. This suggests that the improved moisture balance resulted in an increase in fruit set. An antitranspirant applied later, at full expansion of spring flush leaves, increased both the leaf water potential and leaf temperature but caused fruit drop. The increased fruit drop was probably due to a reduction in the photosynthate supply which resulted from the restriction of CO<sub>2</sub> exchange by the antitranspirant film and to reduced carboxylation



activity by excessively high leaf temperature. These deleterious effects did not result from the early-applied antitranspirant because the film cracked with the expansion of the leaves and ambient air temperatures were considerably lower.

In the second study, plant water balance as estimated by leaf water potential, leaf stomatal diffusive resistance, and moisture loss from young fruits varied among the 3 cultivars. Satsuma mandarin suffered the greatest water stress and lost more water from the fruit followed in order by navel and 'Valencia' oranges. The levels of leaf water stress and fruit moisture loss were inversely related to their respective stomatal densities. The data, however, were not compatible with the assumption that greater water deficits were associated with lower fruit set. Navel oranges, which have a history of low fruit set, had better plant water status than the satsuma mandarin, which has a record of higher fruit set. It is possible, of course, that navel oranges might have a different moisture status requirement for their basic physiological processes than does the satsuma mandarin.

## INTRODUCTION

Navel oranges are noted for their superb quality and they constitute the major portion of plantings in regions favorable to their production. Unfortunately, navel oranges yield very poorly in many regions of the world, including Florida. The cause of poor fruiting is not well understood even though considerable attention has been given to the problem.

Fruits of most horticultural varieties develop from ovaries which enlarge as a result of hormonal stimulation accompanying the sexual process and subsequent seed formation. Fruits, without this stimulus usually fall at an early age, except for parthenocarpic cultivars, i.e., those that have the ability to set and mature fruit without sexual fertilization.

Navel oranges have sterile ovules and pollen thereby precluding the sexual process (162, 219, 220), and they are not strongly parthenocarpic (39, 162, 219, 220). They shed fruit during times of environmental stress such as a deficiency of water (38, 33, 101). Coit and Hodgson (39), as early as 1919, noted that navel fruit drop was unusually heavy in hot dry areas of California. They attributed this to moisture stress, pointing out that less drop occurred in cooler coastal areas. Others have also related fruit drop to moisture stress (54, 56, 107, 212).

Parthenocarpic cultivars appear to suffer more from moisture stress than do seedy ones. Koo (121), for example, showed that yields of

'Marsh' grapefruit, 'Hamlin' orange, and 'Valencia' orange, all had greatly increased yields as a result of irrigation but the yields of the seedy 'Pineapple' orange were only slightly increased. Even so, many seedless cultivars fruit better than navels.

Experience from application of water in commercial groves has indicated that irrigation alone is not a complete solution to the problem. Castle et al. (31) have emphasized the difficulty in maintaining an adequate moisture level in the deep, sandy soils of Florida.

Recent work in California has shown some success in improving fruit set of navels through evaporative cooling with intermittent overtree sprinkling (26).

Work by Albrigo (4) and Albrigo et al. (10) indicated that the application of Vapor Gard (pinolene), a polyterpene antitranspirant, at low concn, could successfully improve the water status of citrus trees.

The purpose of this research was threefold. First, work was conducted to determine whether reducing moisture stress through the application of a water mist with overhead sprinklers and through the use of antitranspirants applied with a high pressure sprayer, would reduce fruit drop. Second, experiments were performed to determine whether leaf water deficits and fruit moisture losses from high yielding commercially seedless cultivars, such as satsuma mandarin (C. unshiu Marcovitch) and 'Valencia' orange (C. sinensis [L.] Osbeck) would differ from those of the light bearing navel orange. Third, stomatal density and dimensions for the above citrus cultivars were measured to determine whether these factors influence tree water balance.

## LITERATURE REVIEW

### The Fruit Set Problem

Navel orange cultivars have in common the presence of the navel, a rudimentary secondary fruit embedded in the apex of the primary fruit, and the commercial seedlessness (102). Seedlessness in navels results because no functional pollen is produced (162, 219, 220) and nearly all of the megaspore mother cells or embryo sacs degenerate so that only occasional viable ovules are formed (162). Production of a few seeds is possible by cross-pollination (219, 220). Fruit setting is distinctly parthenocarpic (39, 68, 219, 200).

On the other hand, low productivity resulting from excessive shedding of young fruits has been a major problem with navel oranges in different citrus areas (39, 55, 62, 79, 219). Weak parthenocarpy and greater sensitivity to environmental stresses have been indicated as possible reasons for the poor fruiting behavior (39). Coit and Hodgson (38, 39) reported an excessive drop of young fruits of 'Washington' navel oranges grown in hot and dry interior valleys of California and Arizona. They indicated the productivity in those areas represented only 25% of that in cooler, coastal regions. They pointed out that the abscission of flowers and fruits, under an unfavorable environment, was more severe in navel oranges than in seedy or less sterile varieties. Platt (167) observed that adverse meteorological conditions in 1957 resulted in greatest abscission of leaves and young fruits in navel

oranges, followed in descending order by 'Valencia' orange and grapefruit. In Florida, navel oranges are known as light bearers, having large fruits which tend to dry out or split, especially if the navel is excessively developed. Fruit drop is severe during periods of moisture depletion, which commonly occur in the deep, sandy soils during the spring (79, 129, 219).

Navels have an unusually heavy bloom. Bain (14) observed that the amount of bloom and the shedding of flowers and fruits is directly proportional to length of the dormant period preceding flowering. Moss and Muirhead (156) noted that the major component of yield in citrus is the no. of fruits harvested, which is related to the no. of flowers formed and the proportion of flowers that set fruit. Van Noort (212) and Moss (154) indicated that % fruit set was lower when a large no. of flowers was initially present on the plant, but there were more fruit per tree.

In citrus, the response to an abundant bloom is mainly through fruit abscission rather than a reduction in fruit size (65). Bowman (24) concluded an adequate commercial crop will develop of 1% of the flowers produce fruit. Erickson and Brannaman (65) reported only 0.2% of navel flower buds produced fruit as compared to 1% for Valencia. They also reported the greatest amount of abscission occurred with flowers and fruit of the smallest size group with progressively less abscission as size increased. The heavy natural thinning of navel orange fruits is possibly one of the reasons for their large size. Artificial thinning in citrus has resulted in increased size of the remaining fruit (96, 104).

Varying degrees of alternate bearing are known to occur in all citrus cultivars. Jones and Cree (112, 113) indicated 'Washington'

navel and 'Valencia' oranges both had a marked tendency toward alternate bearing, but the intensity of alternation was considerably less in navels, probably due to their earlier harvest.

In navel oranges, the abscission of reproductive structures takes place throughout the season, however, 3 more or less defined waves or periods of drop are recognized (35, 39, 65, 138). Postblossom drop, a heavy bloom in March is followed by an intensive drop of flower buds, flowers, and young fruits, which extends to the next 3 weeks after the petals fall. The greatest amount of abscission takes place in the first wave. This is a natural thinning process which is mainly a result of competition for food, since a tree cannot bring to maturity all the potential fruit formed during flowering (65, 138). The second wave of drop occurs from early May to mid-June and is called "June drop" (33). This drop is usually the one most closely related to the final yield and it is largely the result of an abnormal sensitivity of the abscission mechanisms to unfavorable environmental conditions (39, 113, 138, 167). The third wave is the preharvest drop which takes place when the fruits have almost reached their final size. It has been indicated that the preharvest drop is probably due to the decline in vigor of the fruit with maturity and to the activity of Alternaria citri (35, 39).

Attempts to improve fruit set and yield of navel oranges have been made through fertilization, girdling, cross-pollination, and application of growth regulators. Only limited success has been achieved.

## Efforts to Improve Yields

### Mineral Nutrition

High yields in citrus can be expected only if a proper fertilization program is followed. Citrus yields are more directly related to N than to any other essential element. Its deficiency greatly reduces fruit production (33). Most results point to a high N demand during blossom and immediately afterwards. Chapman and Parker (34) noted a shortage of N is most critical just prior to, and during blossoming, fruit setting, and "June drop." Bouma (23) indicated that insufficient supply of N during this stage can result in excessive shedding of flowers and immature fruit in navel orange. He suggested fertilization should be given at a time to provide sufficient N for early spring growth and fruit set.

Reduced "June drop" has been achieved with applications of N fertilizers in the spring months (201). Lenz and Cary (137), working with potted navel trees developed from stem cuttings, showed a better fruit set as a response to adequate levels of N. Villiers (213) reported considerable increases in yields of navel and 'Valencia' oranges with N fertilization, the better responses being obtained on sandy soils in subtropical climates.

### Girdling

Girdling is an old practice claimed to improve the setting of parthenocarpic fruits. In most instances, it consists of a single, knife cut, entirely around the tree trunk, through the bark and into the young wood. The effectiveness of the practice has not been established for all citrus varieties on which it has been used.

In Israel, girdling 'Clementine' tangerines has produced inconsistent results (160). Gonzales-Scilia (86) reported from Spain that girdling selected branches of the tree instead of the trunk resulted in increased fruit set of 'Clementine.' With 'Shamouti' orange, Aharoni et al. (3) noted a significant increase in yield with girdling for one season. Krezdorn and Wiltbank (132) indicated 'Orlando' tangelos girdled for 8 consecutive years produced significantly more fruit than control trees without evidence of tree damage or alterations in fruit size and quality. It was suggested that the extreme vigor of 'Orlando' tangelo favored successful fruiting.

Most work on girdling of navel oranges shows increased fruit set in the first year with a gradual reduction in subsequent years (11, 143). Allwright (11), in S. Africa, reported that girdling in one year promoted yield increases in navels of 8.1 to 20.4%, but girdling in 4 successive years resulted in a decrease of 5.5% as compared to untreated trees. Others (183, 184, 185) have reported similar results. Rackham (172), on the other hand, working with moderate to high vigor navel trees, failed to observe any yield reduction trend or adverse effects on the trees, due to girdling, in 7 years of tests. In other instances, girdling has been indicated as ineffective in increasing yield of navel oranges (13). In Florida, Krezdorn (128) obtained initial success in girdling 'Dream' navel oranges. He indicated, however, that the volume of negative results obtained elsewhere and the modest vigor of navel trees make unlikely a continued successful girdling of this cultivar.



### Cross-Pollination

Attempts to improve fruit set of navel oranges by cross-pollination date back to 1894 (219). After a series of experiments in Florida, in which navel orange flowers were crossed with pollen from different citrus cultivars, Webber (219) observed a slight increase in seediness but no apparent effect on fruit setting. Later, in 1930, he indicated that pollination by insects would not likely have any considerable effect on the crop of navels in solid plantings (220). Similarly, Coit and Hodgson (39) concluded that the setting of fruit in navels is entirely independent of pollen.

Some studies have shown increased fruit set in navel oranges when flowers were hand cross-pollinated (55, 62, 63, 168). Pomeroy and Aldrich (168) reported an increase in fruit set of navel orange hand cross-pollinated with 'Pernambuco' and 'Foster' grapefruit. They indicated that this response could be a result of stimulatory effects of pollination. El-Tomi (62, 63), in Egypt, observed, in addition to production of seedy fruit, a significant effect of hand-pollination in minimizing the "June drop" losses of navel orange.

The positive results obtained with cross-pollination have not been convincing enough to recommend this practice for improving fruit set of navel oranges. More recently, Krezdorn (129), using 3 different sources of pollen, found virtually no effect of crossing in the fruit set of 'Dream' navel orange. Soost (191, 192) noted that the presence of seeds in the parthenocarpic fruit would possibly reduce the sensitivity of these cultivars to fruit drop under adverse environmental conditions. Since navels have few viable ovules and in consequence, few seeds are set, regardless of the pollen source, it is unlikely that cross-pollination

under field conditions would increase yield to a significant level (129, 191, 192).

### Growth Regulators

Plant growth regulators have been associated with different stages of fruit development in citrus and different compounds have been tested in the control of fruit abscission (37, 92, 139, 225).

Auxin compounds like 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been successfully used in reducing or delaying the preharvest abscission of fruits of different citrus cultivars, including navel oranges (52, 64, 80, 111, 195, 196, 197). In California, the application of 2,4-D at very low concn has become a commercial practice in the reduction of preharvest fruit drop, which accounts for considerable losses especially during the latter part of the harvest season (111).

With respect to fruit set most of the information available indicates that application of auxin compounds have been relatively ineffective. Some success has been achieved with gibberellic acid (GA) (93, 131, 193). Several trials in California and Florida have demonstrated the positive effects of GA and potassium gibberellate (KGA) in increasing fruit set of citrus when applied to branches or to individual clusters of flowers or small fruits (93, 94, 131, 193). Application of GA or related compounds to entire citrus trees have, however, given negative results in most cases (95, 129, 130, 131). Hield et al. (95), in California, conducted a series of trials in which different concn of KGA were sprayed over entire trees of 'Washington' navel oranges. They found growth regulators failed to improve fruit

set at low concn. Phytotoxic effects like leaf drop and twig dieback, and reduced yields were observed in some experiments at high concn. In Florida, Krezdorn (129, 130) and Krezdorn and Cohen (131) reported increased fruit set of 'Orlando' tangelo trees sprayed with GA; however, GA concn as low as 50 ppm were sufficient to cause severe leaf drop and to produce abnormal fruits. With navel oranges, GA concn of as high as 500 ppm failed to cause apparent phytotoxic effects but it was also ineffective in promoting fruit set, even though excellent results were obtained when individual young fruits were dipped in high concn of GA.

### Environmental Stresses

#### Influence on Fruit Set

Considerable attention has been given to the effects of environmental stresses on the set and subsequent development of navel orange fruits. Available evidence indicates the excessive abscission of young navel fruit is largely a result of environmental stresses, especially water deficits and high temp prevalent during postblossom and fruit-setting periods. Improvement of fruit set through the alleviation of water and heat stresses has become a promising line of research.

As early as 1917, Hodgson (101) reported an unusual shedding of young navel fruits which was more severe in areas where low precipitation, low humidity, high temp, and hot dry winds were prevalent. Coit and Hodgson (38, 39) indicated that pronounced daily water deficits developed in fruits and leaves of navel orange during the hot growing season in the drier regions of California. They noted the leaves could actually withdraw water from the fruit and the stress thus developed was

the stimulus to premature fruit drop. Many other studies pointed to water stress as the apparent cause of most early fruit abscission and the importance of keeping an adequate soil moisture level during fruit setting was often emphasized (53, 54, 56, 70, 88, 107, 109, 122, 188, 206, 212).

Haas (88) indicated that in evaluating the effect of water stress on fruit abscission the evaporation from the fruit itself should also be considered. He observed that the amount of fruit drop declines as the fruit increases in size. This was attributed to the fact that the % loss of moisture from young fruits decreases rapidly as the fruit increases in size, which is largely a result of the decrease in proportionate surface of evaporation. Similarly, Coit and Hodgson (39) noted max shedding takes place when young fruits are from 0.5 to 2.0 cm in diam and very little drop occurs after they reach a 5.0 cm diam. The period of max fruit drop corresponds approx to Stage II of fruit development, as defined by Bain (15), in which marked cell enlargement and fruit growth take place. In this stage, environmental factors have the most pronounced effect on growth and development (144, 216, 217). Adverse conditions halting fruit growth at this time may result in abscission.

Some evidence is available to indicate a redistribution of water between organs within the tree can take place which may alleviate the severity of drought conditions (194, 206). It has been stated that under certain conditions fruits may act as a reservoir of water for the rest of the plant (18, 32, 39, 101). Spurling (194) and Trumble (206) noted that well-developed fruit on the tree may act as a buffer when the plant is suffering from a severe water stress. They indicated this might be the reason leaf and fruit drop is more severe for navel than for

'Valencia' orange which carry the old crop when new fruit is being produced. In contrast, Rokach (178) and Kaufmann (116) showed that water is withdrawn from the peel rather than from the pulp of the fruit. Therefore, the fruit may not be as large a reservoir of moisture as it appears to be. In the same connection, West and Barnard (221) related an experience in which 'Valencia' orange trees suffering severe drought showed immediate improvement in color and turgidity of the leaves when the fruit was removed. Kaufmann (117) and Elfving and Kaufmann (60) noted that changes in water content of some fruits are governed largely by fruit transpiration and that no convincing evidence was available to indicate that a transpiring orange fruit on an undisturbed branch of the tree could act as a midday reservoir of water for the leaves.

The water status of citrus trees have been properly assessed through determinations of water content (39), water saturation deficit (40, 202), water potential (8, 19, 60, 115, 119), and stomatal diffusive resistance to water vapor (8, 60, 164). Early in 1917, Hodgson (101), by determining moisture content of orange leaves and fruits, observed that these organs undergo a diurnal decrease in water content. The lowest moisture level was reached in the afternoon between 1 and 4 PM and fruits had a higher water content than leaves at all times. Oppenheim (159) observed that stomata of sour orange begin to open early in the morning, reach a max aperture by 10 AM, remain fully open until 4:20 PM, and close at about sunset. Recent studies indicated that both leaf and fruit water potentials undergo diurnal fluctuations with min values being reached in the early afternoon. Fruits show less pronounced cycling than leaves (8, 60, 61). Typical fluctuations in water potential tend to be accompanied by simultaneous changes in stomatal diffusive

resistance and transpiration (8, 27, 60, 61). The less pronounced variation in fruit water potential during the day has been attributed to its higher water vapor diffusive resistance (8, 60). It is well known that citrus fruits are subjected to diurnal expansion and contraction, the degree of variation in size being dependent upon soil water availability and evaporative demand (60, 91, 97, 99, 140, 204). The reversible shrinkage was found to be related to simultaneous changes in fruit and leaf water potential and fruit diffusive resistance (60).

Elfving et al. (60, 61) have suggested that soil temp, vapor pressure deficit (VPD), and soil water supply are the 3 major environmental factors affecting water stress in citrus leaves. Extremes of temp may increase the resistance to water flow in the root system which can result in reduced leaf water potential, especially if the evaporative demand is high (21, 28, 38, 60).

High VPD and consequent high evaporative demand cause a more negative leaf water potential (60). Palmer et al. (164), however, found that after VPD reaches a certain high level, leaf water potential starts to increase as a result of higher stomatal resistance. Elfving et al. (61) indicated leaf water potential is related to the transpirational flux, which is defined as the ratio of VPD to leaf resistance. They noted that leaf water potential decreases in a curvilinear fashion with increasing values of VPD/leaf resistance under nonlimiting conditions of soil water and temp. Under limiting environmental conditions, however, leaf water potential can become more negative than predicted by the ratio.

Soil water supply markedly affects the water status of citrus trees. Highest values of leaf water potential are reached immediately

after irrigation, becoming more negative as soil moisture is being depleted (8, 60, 61, 140). Under limiting soil water supply, complete nighttime recovery from daytime water deficit can be prevented. This is indicated by more negative sunrise leaf water potentials (8, 60, 61). Limiting soil water can cause internal plant water deficits that result in increased leaf resistance to diffusion of water vapor (72). Kriedemann (135), by withholding water from potted orange trees, obtained a wide range of stomatal apertures. He found that variation in leaf resistance resulting from moisture status or cyclic oscillations in stomatal aperture was related to changes in photosynthesis and transpiration.

A considerable amount of evidence points to water stress as the major, primary cause of fruit drop in navel oranges, but it is apparently not the only factor controlling the process. High temp alone or a combination of moisture stress and high temp have also been indicated as causal factors of early fruit abscission (98, 108, 113, 117, 154, 164). Since high temp are frequently accompanied by high plant water deficits, it has been difficult to separate the effects of temp from those of water stress. Multiple regression studies have shown a negative correlation between max temp during early fruit development and yield of navel oranges (98, 108, 113). Moss (154), working under controlled conditions, reported that citrus fruit drop was a direct result of high temp rather than an effect of low humidity causing internal water stress. Similarly, Furr et al. (71) failed to show soil moisture depletion as the cause of excessive fruit drop. They pointed out high temp rather than water deficit was the major factor determining young fruit abscission of navel oranges. Reuther (176) also emphasized the effect of high temp in promoting citrus fruit drop but he noted the

separation of direct effects of high temp from those of temp promoting water stress in the plant has not been possible.

The determination of the ultimate relation between environmental stresses and fruit abscission has been a difficult problem, resulting in much speculation. It has been indicated that moisture stress results in decreased cytokinin activity, and increased abscissic acid levels and ethylene release (2, 106, 163). Such changes can help the plant in adapting to moisture stress but can conceivably lead to abscission of plant parts. Little work, however, has been done relating moderate levels of moisture stress to hormonal balance and abscission of fruit (106, 163).

On the other hand, early workers offered suggestions based on the presumption that high rates of photosynthesis and CHO accumulation were conducive to adequate setting and subsequent development of the fruit. Accordingly, it was thought fruit drop was a result of nutritional disturbances, such as intense competition for nutrients, and shortage of CHO or other essential metabolites, that were caused by high temp or a combination of high temp and water deficits (14, 71, 113, 154). In agreement with the general idea, thinning of flowers and young orange fruits has resulted in increased size of the remaining fruits and higher yields in the following season (96, 165, 221, 222). In oranges, later blossoms have been shown to have better chance to set fruit, which is attributed to the fact they are less subjected to the intense competition of the new spring flush of growth for stored food (89). Also, leafy inflorescences set a higher % of fruits than leafless inflorescences (174). Convincing evidence has been presented indicating that new leaves influence fruit set by providing photosynthate to the adjacent developing



fruit (134, 153, 157, 171). Powell and Krezdorn (171) working with calamondin (*C. madurensis* Lour.) showed that self-pollination and GA application caused strong mobilization of  $^{14}\text{C}$ -metabolites to the young developing fruit which they noted was essential for fruit set and development.

In spite of relatively high stomatal density and abundant chlorophyll, orange leaves have low photosynthetic capacity (133). Thompson et al. (205) demonstrated that  $\text{CO}_2$  exchange in leaves of rough lemon was reduced by half when leaf temp of the plants at 19% relative humidity was increased from 22° to 34°C. A smaller effect in photosynthesis was observed at higher relative humidities (30 to 54%). Kriedemann (133) and Possingham and Kriedemann (170), working with sweet orange and lemon, reported similar findings. They found a sharp optimum of photosynthesis occurred between 15° and 20°C in dry air but at relative humidities higher than 80%, net photosynthesis peaked at 30°C. There was little reduction at 35°C. The enhancement of photosynthesis at higher relative humidities was interpreted as a result of reduced mesophyll and stomatal resistances to diffusion of  $\text{CO}_2$  (133, 170, 205). Recent work with navel oranges indicated high temp and high VPD caused increased leaf resistance with a subsequent decrease in the gross uptake of  $^{14}\text{CO}_2$  (164). Also, a restricted flow of photosynthate to young fruit occurred during heat stress. The authors proposed that the induction of early fruit drop in navel orange is brought about by physiological events stimulated by a combination of low soluble food reserves, low photosynthesis, and high food demand by developing leaves and young fruits. Finally, in a more direct approach, Moss (155) demonstrated the application of photosynthetic inhibitors during early fruit development caused a significant reduction in fruit set of navel orange.

## Modifying Environmental Stresses

### Misting

Attention has been called to the fact plant water stress depends upon relative rates of water absorption and water loss rather than on soil water supply alone (123, 127). Water deficits caused by excessive transpiration have resulted in many kinds of plants making more growth at night than in daytime (106, 124, 127, 141).

As early as 1954, it was shown that sprinkling during the hottest hours of the day caused substantially higher rates of plant growth than the same amount of water applied to the soil (199). In 1957, Langhans (136) reported that rose plants intermittently sprayed with a fine mist of water had lower leaf temp, negligible transpiration losses, and higher rates of plant growth.

Most studies point to mist irrigation as an efficient method of modifying the microclimate of the plant canopy. It has been shown to reduce soil, air, and leaf temp, to increase relative humidity, wet bulb temp, and vapor pressure. Reduced rates of transpiration and increased yields have been the most common benefits of such modified environmental conditions (22, 29, 85, 177).

Mist irrigation during periods of high impinging solar radiation and high temp has caused significant yield increases of several vegetable crops. Carolus (29) reported that increased irrigation frequency resulted in an increase of strawberry yield of 200 crates per acre. The misted fruit had a substantial increase in quality, a size increase of 85%, brighter color, and better storage characteristics than the controls. Yield and quality of tomatoes have been enhanced by sprinkling

irrigation at times of high atmospheric stress (30). Yields of snap beans were increased by up to 100% (223). Howell et al. (105) were among the first to show the effectiveness of mist irrigation in increasing leaf water potential. The alleviation of water stress resulted in yield increases of southern peas (Vigna unguiculata) of up to 60%. Bible et al. (22) reported mist irrigation lowered plant temp by more than 9°C and increased the yield of muskmelon by 30%, cucumbers by 70%, and tomatoes by 30 to 50%, but dates of maturity of all crops were delayed. They postulated that misting, by decreasing tissue temp and water stress, reduced transpiration rates and kept leaves turgid and stomata open, thus increasing net photosynthesis.

Miller et al. (150) working with avocados showed overhead sprinkling reduced surrounding air temp by 3 to 4°C and increased relative humidity by 6 to 12%. Sprinkling was suggested as a possible means of reducing premature fruit drop in the summer or at fruit-setting time in the spring.

Other fruit crops have shown positive responses to mist irrigation. Overhead sprinkling of cherries at midday during the stage of fruit maturation sized a heavy crop and resulted in more uniform fruit maturity (16). Mist irrigation has been effective in increasing fruit size of 'Red Delicious' apples (210) and 'Early Amber' peaches (179). Very recently, Brewer et al. (26) obtained a significant reduction in early fruit drop of navel oranges by intermittent sprinkling during stress periods. At higher rates of water application increases in fruit set of 35% and yield of 40.5% were reported.

Most of the early work with mist irrigation was directed at reaching the max microclimate modification that could be obtained with the system.

Frequently, evaporation cooling was provided by sprinklers operating continuously without much regard to efficient water use. Continuous sprinkling was soon evaluated against sequential operation. Chessness and Braud (36) operating sprinklers in a sequential fashion found the max microclimate modification occurred within 15 min after the sprinkling had been initiated. This indicated an irrigation cycle of 15 min on and 15 min off would be appropriate. Gilbert et al. (85) observed that 82.5% of the cooling was obtained during the first 3 min of sprinkling operation. Fifteen minutes with the sprinkling off were required to return leaf temp to its original level. A sequential operation of 3 min on and 15 min off was suggested. Sequential operation of sprinklers was shown to be nearly as effective as continuous sprinkling in both investigations.

#### Antitranspirants

The possibility of retarding transpiration and improving plant water status through the application of certain chemicals is an old idea (76); however, only in recent years have antitranspirants been the object of intense investigation, considerable attention being given to their agronomical use (44, 73, 74, 214). A general review of plant antitranspirants was made available by Gale and Hagan (74), in 1966. The theories concerning their mechanism of action and effects on plant growth were extensively reviewed by Waggoner (214) and Zelitch (229). Since then, new chemicals capable of reducing plant transpiration have become available, and the results of several studies on their effects and problems involved in their application have been reported (1, 4, 9, 25, 45, 48, 50).

Different kinds of materials with antitranspirant properties are available (73, 74). This review will be concerned with emulsions of wax, latex, or plastics, which when sprayed form a thin, discontinuous, water-vapor-restricting film over the plant surfaces. Most recent work has been with such materials (4, 47, 50, 73, 74, 76, 214).

Film-forming antitranspirants have had a variety of applications and many potential uses are yet to be explored. They have been largely used to prevent the desiccation of plants during transplanting operations. Vapor Gard (polyterpene), Mobileaf (wax emulsion), and CS-6432 (wax-latex emulsion), sprayed over young navel orange trees before transplanting, effectively increased leaf water potential, thereby reducing the stress of transplanting (45). Similarly, the immersion of aerial portions of the tomato seedlings in antitranspirant emulsion has increased the % of plant survival after transplanting (67).

A preharvest spray of antitranspirant has been shown to increase stomatal resistance to water vapor diffusion thereby raising the plant water potential. Such treatment has improved fruit size of peach (17, 43, 48), cherry (46), and olive (49). The increase in fruit vol was invariably accompanied by a dilution of soluble solids. Applying antitranspirants too early resulted in decreased dry wt per fruit in some instances (46). Davenport and coworkers contended that, because the antitranspirant coating slowed transpiration and photosynthesis, trees should be sprayed at a time fruit growth is more dependent on a high water potential than on the accumulation of photosynthates (43, 46, 49). They also indicated enhanced fruit growth was primarily a response to antitranspirant leaf coating rather than a direct effect of fruit coverage.

The coating of oranges with Tag, a polyethylene-wax emulsion, has improved the appearance of fruit and extended their storage life by about 100% (20). Albrigo and coworkers found a preharvest spray of Vapor Gard (Pinolene), resulted in improved peel quality of oranges at harvest and during storage. This was attributed to reduced peel dehydration and possibly to decreased fruit respiration (9, 10).

Gale et al. (77) reported a monthly spray of Tag caused a 21 to 44% reduction in water uptake by bananas without noticeable effects on growth or yield. Applications of Tag and S789 (latex emulsion), 1.5 to 3 months before harvest, resulted in a 2 to 3 week delay in the ripening of grapes, which was attributed to the improved water balance of the vines (77). Under nonirrigated conditions, yields of antitranspirant-treated vines were increased by as much as 14.5%. The increased yields reported in the literature have been the result of increased fruit size (46, 49, 77). No reference is made of increased fruit set through the alleviation of water stress with film-forming antitranspirants.

One of the imperfections of the early antitranspirants was the inability of the compounds to form an enduring cover on the leaf surface. Emphasis has been given to the importance of antitranspirant films that remain elastic and resistant to degradation (44, 74, 78, 214). Albrigo (4) found that Vapor Gard (Pinolene) formed a film which improved the appearance of nonirrigated 'Valencia' orange trees, and showed little degradation 6 months after the application. Such an antitranspirant could be suitable for long term control of plant water stress.

Negative results with the use of film-forming antitranspirants have also been reported. Fucik (69), using Foligard (acrylic copolymer),

and Goren et al. (87), using Ressec (polyvinyl acetate emulsion), failed to show any improvement in the survival and growth of newly planted citrus trees. Possingham et al. (169), testing several antitranspirants, found that only Acropol (S789) was effective in reducing transpiration but at the expense of reduced photosynthesis and dry matter accumulation. Slatyer and Bierhuizen (189) found a vinyl resin decreased transpiration of cotton plants, but photosynthesis was reduced somewhat more. Very recently, Moss (155) observed that 2 applications of a film-forming emulsion of Acropol at 24 and 38 days after full bloom decreased the photosynthetic rate of navel orange which was accompanied by a slight reduction in fruit set.

One possible detrimental effect of the reduction of transpiration is the expected increase in leaf temp which could result in reduced metabolic activity and consequent growth retardation. Inconsistent results have been obtained as to the effects of transpirational cooling on leaf temp. Antitranspirant treatment has been reported to raise leaf temp by as much as 5°C (41, 224). Slatyer and Bierhuizen (190) noted 50% suppression of transpiration raised cotton leaf temp by 4°C. On the other hand, Gale and Poljakoff-Mayber (75) found little difference between temp of treated and untreated leaves of a no. of species, in spite of a 30% transpiration reduction caused by the treatment. It has been suggested that transpiration is not essential to cool the leaf and other forms of heat dissipation might become more effective if it is impeded (44, 75, 181). Gates (83) suggested most of the existent discrepancies are due to the fact leaf properties and the environment surrounding the leaf have not been properly assessed. He developed a heat budget analysis by which leaf temp and transpiration could be

evaluated over different leaf characteristics and environmental factors. He stated evaporation of water from a leaf must affect its temp (84). Conditions exist under which variations in transpiration rate may make relatively little difference in leaf temp. Leaf temp, however, can be substantially raised with a decrease in transpiration under conditions of high impinging radiation, high temp, and low wind (83, 84).

The basis for the use of antitranspirants lies in the principle that transpiration can be decreased naturally or artificially with a relatively smaller decrease in photosynthesis (74, 214, 230). Photosynthesis as a diffusion process differs from transpiration by the inclusion of mesophyll resistance to  $\text{CO}_2$  ( $r'_m$ ). Thus, stomata diffusion resistance forms a greater part of the total diffusion pathway for transpiration than for photosynthesis. Logically, an antitranspirant film will reduce transpiration proportionally more than photosynthesis, if the material does not increase  $r'_m$ , and if the film resistance to water vapor diffusion is greater or at least equal to its resistance to  $\text{CO}_2$ . Present evidence indicates that the available film forming materials do not provide that kind of selective permeability to gases ( $\text{CO}_2$ ,  $\text{O}_2$ , and  $\text{H}_2\text{O}$  vapor) (76, 226). Gale and Poljakoff-Mayber (76) noted that polyethylene, the most promising material, is 4 to 5 times more permeable to water vapor than to  $\text{CO}_2$ . Waggoner (214) reported that the resistance to  $\text{CO}_2$  relative to water vapor ranges from about the same for polyethylene to at least an unfavorable 10,000 for cellophane. He also stressed the fact these materials have a large absolute resistance to  $\text{CO}_2$  diffusion. A 1  $\mu\text{m}$ -thick film of the most permeable material can impose a resistance to  $\text{CO}_2$  diffusion twice as great as that found in a natural leaf, which would result in a drastic reduction of photosynthesis.



In view of the present considerations a question is posed of how can a favorable photosynthesis/transpiration ratio be achieved after an antitranspirant treatment. For one thing, the amount of  $\text{CO}_2$  needed for photosynthesis is considerably smaller than the water vapor lost by transpiration and part of the  $\text{CO}_2$  required comes from respiration. According to Waggoner (214) the answer to the question may lie in the imperfect coverage of the leaf surface by the antitranspirant film. His view is that the plastic compounds form films which only partially cover the stomata-bearing surfaces, especially under field conditions. Under conditions of high transpirational demand, an untreated leaf would soon wilt and close its stomata while a partially covered leaf would continue to transpire at a low rate, remaining turgid with the stomata open. Thus, a decrease in transpiring surface per plant can avoid wilting, and even increase long term transpiration, while allowing photosynthesis to continue. It has also been indicated that with the increased hydration a lower  $r'_m$  and greater photosynthetic ability may be expected which would allow a more favorable photosynthesis/transpiration ratio (133, 214). Findings obtained from field experiments and tested models have agreed with the proposed explanation (73, 74, 76, 77, 142, 214).

Waggoner's view is shared in total or in part by other investigators. Davenport et al. (43) indicated the partial leaf coverage by the antitranspirant film causes an increased leaf water potential which leads to an increase in the stomatal aperture immediately under the film as well as on the uncovered portions of the leaf. In spite of reduction in photosynthesis, increased growth can result from antitranspirant treatment if the process at that time is more dependent on water status

than on CO<sub>2</sub> assimilation. The need for antitranspirant formulations that form an enduring desirable degree of leaf coverage rather than a persistent entire coating has been stressed by Waggoner (214) and others (50, 142).

Gale and Hagan (74) and Davies and Kozlowski (50) pointed out the importance of defining environmental conditions for the interpretation of results obtained from field trials with antitranspirants. Accumulated information indicates the effects of antitranspirants will depend upon the plant species, the properties of the material used, the degree of coverage, and environmental conditions affecting the water status of the plant (50, 73, 74, 214). These factors may be expected to interact and determine the final effect of the antitranspirant treatment.

#### Water Losses and Stomata Characteristics

Stomatal diffusive resistance and rates of water loss show considerable variation among and within plant species. Since large differences in stomatal resistance may determine differences in net photosynthetic rates (103, 135), water use efficiency or ratio of transpiration to net photosynthesis also varies markedly among plants. Such variation has been shown within different genera and species (57, 81, 151), including Citrus (118). These principles are useful in the identification of adaptive mechanisms and constitute the basis for development of plant varieties with high rates of net photosynthesis and adequate regulation of water loss (81, 118, 151).

Plant water losses are frequently related to stomatal densities and dimensions. It should be recognized, however, that leaf morphological characteristics are only one factor determining variation in

water relations among plants. A more important role can be played by the inherent plant ability in controlling stomatal aperture (126, 203, 215) and by differential stomatal response to environmental conditions (51, 126). Other factors that may be involved include leaf shedding, microphyllly, epicuticular waxes, and root/shoot ratio (126).

Marked variation in frequency and size of stomata occur not only among different varieties of the same species, but also in the same variety, or between parts of the same individual plant subjected to the influence of different environmental conditions (58). Plants grown under conditions of high illumination and water shortage normally show higher stomatal frequency (145, 173). Similarly, there are more stomata per unit area of sun leaves than of shade leaves and more in the upper as compared to lower leaves of the same shoot (145, 173, 228). The vertical gradient in stomatal density between lower and upper leaves has been attributed to the increase of water deficiency in the upper leaves (198, 228). Increased no. of stomata per unit area has been indicated as resulting from limited enlargement of epidermal cells, including guard cells (180, 218). Water shortage and high light intensity limit expansion of the leaf surface via cell enlargement and favors the formation of smaller and thicker leaves. In general, stomatal frequency is inversely related to stomatal size (58, 180). The no. of stomata varies among different plant organs, being much less common on fruits than on leaves of most plants (117). Even within a single fruit or leaf, stomatal frequency varies. It is influenced by the distance from the fruit button or styler end (5, 90, 209), by the distance from the leaf sheath and major veins, and by the presence of oil glands (148, 175, 209).

In establishing a connection between plant water balance and leaf morphological characteristics it is worthwhile to refer to xerophytism or adaptation to drought. Many xeromorphic features can develop when plants are grown under conditions of high light intensity and deficiency of water (166, 173). One of the most conspicuous features of xeromorphic leaves is the small ratio of the external leaf surface to its vol (147, 218). The reduction of the external surface area is accompanied by a reduction in cell size, an increase in thickness of cell walls, a more compact network of veins, smaller and more numerous stomata per unit surface area, and strongly developed palisade tissue (145, 161, 218). Not all xeromorphic structures are effective in retaining water. Features such as increased no. of stomata and well developed palisade tissue are conducive to increased transpiration in some instances, especially under favorable conditions of water supply (66, 146, 186). Present evidence indicates many xerophytes grown in humid habitats show higher rates of water loss per unit of external leaf surface than do mesophytes (125, 146, 186, 207). Similarly, higher rates of transpiration have been shown in upper, more xeromorphic leaves than in lower leaves, transpiration being measured under the same conditions of light intensity (145, 207). According to Turrell (207) the higher transpiration rates of xeromorphic structures under humid conditions can be explained in part by the higher ratio of internal to external surface associated with the palisade tissue of xeromorphic leaves.

In connection with stomatal regulation of transpiration, Muenscher (158) could find no significant relationship between the amount of water loss and no. of stomata or length of the stomatal pore; however, Yapp (227) indicated a high stomatal frequency tended to increase the

transpirational capacity of the plant. Kozlowski (126) noted that small, few, and sunken stomata constitute an important leaf adaptation for avoiding drought. Most work with mesomorphic cultivated plants tend to establish a positive relationship between rates of water loss and low stomatal resistance associated with large stomata and/or high stomatal frequency. Dobrenz et al. (57), relating water use efficiency to stomatal density of 6 clones of blue panicgrass (Panicum antidotale Retz.), found drought tolerant clones had fewer stomata per unit area than drought susceptible ones. Miskin et al. (151), working with barley, indicated lines having lower stomatal frequencies had higher stomatal resistances and transpired less than lines with more numerous stomata. Tal (203) reported the wilting tendency of some tomato mutants was mainly a result of wide stomatal openings, resistance to stomatal closure, and high stomatal frequency. Siwecki and Kozlowski (187) concluded that transpiration rates of 6 Populus clones were more closely related to stomatal size and frequency than to internal leaf anatomy. Cold hardiness and the associated ability of the plant to retain moisture was negatively correlated to no. of stomata per leaf area for 11 cultivars of Ilex opaca (120).

In citrus, variation in transpiration rates as estimated by leaf water potential or actual loss of water, has been reported for a limited no. of scion cultivars grown on different rootstocks (19, 42, 78, 149) and for different scion cultivars grown on the same rootstock (118). Very little has been done to establish a relationship between plant water balance and leaf characteristics. Hirano (100) tried to establish an association between stomatal frequency of various species of Citrus and related genera with their place of origin. He concluded that most

species originating in the tropics had higher stomatal frequency than those from higher latitudes. It was also observed that hardness was directly related to low stomatal density, a few exceptions being noted.

The stomatal frequency in fruits is normally very low as compared to leaves. The stomatal frequencies of orange and banana fruits, for example, are only about 20% (178, 209) and 3% (110), respectively, of the frequency in leaves. It has been indicated that stomata in fruits do not exert an effective control of gas exchange and water relations, at least not in the same proportion as they do in leaves (110, 117). Haas and Klotz (90) reported water loss from the stem half of mature 'Valencia' oranges exceeded that of the styler half in spite of the greater stomatal frequency of the latter fruit portion. On the other hand, Rokach (178) noted that young 'Shamouti' oranges lost as much water per unit surface area as the leaves.

Thus, a relatively large body of evidence is available indicating the no. of stomata and stomatal dimensions are related to water loss from leaves. The significance of the frequency of stomata of fruits is less certain.

## MATERIALS AND METHODS

### Plant Responses Following Misting and Antitranspirant Treatments

An experiment comprised of 48 approx 10-year-old navel orange trees on sour orange (*C. aurantium* L.) rootstock spaced 4.6 x 3.1 m, on a Kanapaha fine sand, at the Univ. of Florida Horticulture Unit near Gainesville, was conducted in the spring of 1975. All trees received uniform treatment prior to the experiment. This block of trees was divided into 2 equal subblocks of 4, 5- to 6-tree plots, to which treatments were randomly assigned.

Treatment 1 consisted of an application of antitranspirant (Vapor Gard) at petal fall (April 4). Vapor Gard (poly-1-p Menthen-8-9 diyl), a high viscosity polyterpene film former (Miller Chemical and Fertilizer Corp.), was sprayed at 2% v/v and at a rate of 25-30 liters per tree. Treatment 2 consisted of an identical application of Vapor Gard on May 5, after the completion of the spring flush of growth when the leaves were fully expanded. Treatment 3 was a water mist with intermittent overhead sprinkling, initiated April 11 and terminated in mid-August. The water mist was provided by a low angle Rain Bird sprinkler nozzle (Model 1600) mounted at the end of a 2 m PVC riser that extended 0.3 m above the top of the tree. The system operated at a pressure of  $20.78 \text{ Newton/cm}^2$  (30 PSI), providing water mist over each tree at an approx rate of 11.4 liter/min in continuous operation. The sprinklers were cycled by a 100 EF Rain Bird remote control valve connected to a Hewlett-Packard

digital computer. A program was set to activate the system whenever the ambient air temp outside the mist reached values above 27°C. The sequencing cycle was 30 sec on and 10 min off. The system was automatically shut off when ambient air temp dropped below 27°C. Treatment 4 consisted of the control or untreated trees.

Twenty twigs with fruit were randomly selected from the periphery of each tree and tagged. Fruits were counted monthly to calculate the % fruit remaining. The first counting was made on April 30, at the time the initial postblossom drop was judged to be completed. Yield data were collected for every plot in early November. Five to 7 samples per treatment (8 fruits each) were taken for determination of the total soluble solids (TSS) of the juice. Percent TSS was determined with a Bausch & Lomb Abbe refractometer calibrated in °Brix.

Periodic measurements of pressure potential of the leaf xylem sap and stomatal diffusive resistance were obtained from selected trees of each experimental plot to assess the effect of the treatments in alleviating water stress. Pressure potential of the leaf xylem sap is an estimation of the leaf water potential and will be referred to as such. The readings were obtained from single, fully-enlarged, external leaves on the sun-exposed side of the tree during midday hr (11 AM to 3 PM) of clear, sunny days, when water deficits are expected to be most pronounced. Leaf water potentials were determined with a pressure bomb similar to that described by Schollander et al. (1962). The Schollander pressure bomb has been shown to be a reliable means to assess water stress in citrus (19, 115, 119). Stomatal diffusive resistance to gas exchange was measured on the lower surface of the leaves with a LI-60 diffusive porometer equipped with a LI-20s humidity sensor (Lambda Instruments



Corp.). The instrument was used and calibrated according to recommendations of Kanemasu et al. (114), van Bavel et al. (211), and Morrow and Slatyer (152).

Leaf and ambient air temp were measured with epoxy-coated copper-constantan thermocouples. Three thermocouples connected in parallel were affixed to the undersides of leaves inside the first 30 cm of the top of 1 selected tree for every experimental plot. Ambient air temp and solar radiation (obtained with an Eppley pyranometer) were measured in a weather station located 20 m from the orchard. All thermocouple and pyranometer outputs were monitored every 5 min, averaged and printed at the end of every hr of the day by a Hewlett-Packard digital, recorder-computer.

A continuation of the work had been planned for the following season to increase the validity of the results obtained. Frequent winter and early spring freezes resulted in erratic flowering of most navel trees in 1976 and 1977, which precluded conduction of the work as originally planned. Therefore, a modified experiment was conducted in the 1977 season to confirm the adverse effects of a late-spring antitranspirant application on fruit set that were observed in the initial trial. Another objective was to assess more accurately the influence of antitranspirant and misting treatments in alleviating water stress and modifying plant temp.

Trees with the best bloom were selected for this experiment. Vapor Gard (2%) at a rate of approx 38 liters/tree was sprayed on 2 trees on April 25 and again on 7 trees on May 28, when leaves were fully enlarged. The sprinkling treatment was applied to 6 trees and initiated April 23. The system was set to start operating each day, as in the previous

experiment, but a modified working sequence of 1 min on and 10 min off was followed. This sequencing cycle proved to wet the foliage more adequately and to provide better evaporative cooling. The sprinkling was discontinued in early August.

Percent fruit remaining was determined weekly from May 4 to July 26 on the 6 trees receiving the late spray of Vapor Gard, on 6 trees under water mist, and on 7 control trees.

Simultaneous measurements of leaf water potential and stomatal diffusive resistance were taken from east and west sides of selected trees for every treatment. Readings were obtained every 2 hr from sunrise to sunset, 2 to 3 times a week on clear, sunny days, from April 28 to June 21.

Solar radiation, and ambient air, canopy air, leaf, and fruit temp were recorded every hr of the day during the experimental period, with the exception of those times the computer was not working. Thermocouples were affixed with clips and adhesive tape to exposed leaves and fruits, at an approx ht of 1.5 to 1.8 m on east and west sides of the trees. Canopy air temp were sensed with thermocouples placed at about the same ht but 50 cm inside the tree canopy to protect them from direct sunlight. The VDP of the atmosphere was derived from dew point and dry bulb temp to provide an estimate of the evaporative demand and vapor pressure gradient from leaf to air.

#### Water Relations and Stomatal Characteristics of 3 Citrus Cultivars

In 1976, work was initiated to determine possible differences in water relations between 3 commercially seedless citrus cultivars. All work was conducted in a commercial planting near Eustis, Florida, with

'Valencia' orange, navel orange, and satsuma mandarin, on sweet orange rootstock. Navel oranges and satsuma mandarins constitute groups of similar cultivars and identification in Florida is open to question. Thus, they will be referred to in this paper as navel oranges and satsuma mandarins. 'Valencia' orange is a distinct cultivar and will be referred to by that name. Trees of each cultivar or group of cultivars were, however, obtained through vegetative propagation and had identical genotypes, i.e., they represented specific clones. Trees were 15 to 20 years old and spaced 9.2 x 7.6 m. The soil type was an Astatula fine sand and all trees received the same cultural practices.

Simultaneous leaf water potential and stomatal diffusive resistance measurements were taken every 2 hr from sunrise to sunset on east and west sides of trees of the 3 cultivars. Five complete sets of measurements were obtained between May 13 and August 13.

To compare rates of water loss from young fruits, samples of 20 fruits from each cultivar were collected at sunrise, kept cool in ice chests, and immediately brought to the laboratory. Individual fruits were then weighed on a Mettler P163 torsion balance, at intervals, for variable periods of time up to 1 week. The areas of fruit surfaces were calculated from the longitudinal and equatorial diam of each fruit with the use of a program which approximates the fruit to a prolate or oblate spheroid. Fruit surface areas were used in the calculation of the rate of water loss. Samples were collected on 3 occasions in 1976 (6/2, 7/28, and 8/19) and again on 4 different dates in 1977 (5/28, 6/4, 6/18, and 7/23). Thus, fruits at widely different stages of development were used. All fruits were, however, collected in Stage II, a period of rapid cell enlargement (15).

As part of the same investigation, leaf and fruit stomatal frequency and leaf stomatal dimensions were determined for the 3 cultivars. Leaf and fruit stomatal no. were obtained by the replica method of Stoddard (200). A thin film of cellulose acetate (clear nail polish) was painted on leaf and fruit surfaces. Film sections were peeled off after drying at room temp and mounted on glass microscope slides. Cover slips were placed over the sections and held in place with Scotch tape. Leaf stomatal counts were made with a binocular microscope at 400 X which provided a true field area of  $0.1176 \text{ mm}^2$ . Fruit stomatal counts were made at 250 X which provided field of  $0.2827 \text{ mm}^2$ . Several experiments were performed late in the fruit-setting period with young fruits and fully-grown leaves collected from the spring flush. Either 3 exposed leaves or fruits were sampled from east and west sides of the trees in each experiment. Impressions were taken from the widest part of each leaf between the midrib and the margin, and from the areas of the fruit near the styler end. The no. of stomata in 20 random microscope fields was determined from each leaf and fruit. For every fruit sampled, surface area was calculated from equatorial and longitudinal diam. Leaf areas were obtained with an LI-3000 scanning area meter equipped with a LI-3050 A transparent belt conveyer (Lambda Instruments Corp.).

Observations of stomatal dimensions were made with a JEOL Scanning Electron Microscope (SEM). Leaf sections (about  $3 \times 3 \text{ mm}$ ), sampled identically as for stomata counting, were prepared by fixing in 5% glutaraldehyde at pH 7.2 for 1 hr. The tissue sections were then washed in a phosphate buffer and subsequently fixed in  $1\% \text{ OsO}_4$  for 2 hr. After being washed with buffer again, the cut pieces were run through an alcohol-acetone-freon TF dehydration, solvent displacement series and

then critical-point-dried in freon 13 (12). Sections were mounted on stubs and sputter-coated with gold-palladium before examination. Stomatal outer opening dimensions were measured on 540 X SEM micrographs of 7 to 9 random areas of the leaf surface for every cultivar. In order to estimate the depth of the outer chamber, the stomata were first photographed (3000 X) with the surface positived at approx 0° angle; the pore centered in relation to the outer opening and the surface focused in the eccentric plane. The distance a between the left edges of the outer opening and stomatal pore was measured. The angle of observation was then gradually changed until the edge of the stomatal pore was in line (coincident) with the left edge of the outer opening. The tan of the change in angle between the 2 photographs multiplied by the distance a gave the ht of the right triangle or the depth of the chamber.

Analysis of variance, group comparisons, and regression analysis were used, as appropriate, in the statistical treatment of the data obtained from the various experiments at Gainesville and at Eustis.

## RESULTS AND DISCUSSION

### Plant Responses Following Misting and Antitranspirant Treatments

Assuming environmental stresses during the postbloom and fruit-setting periods were the cause of the excessive abscission of young fruits of navel oranges, work was conducted to determine whether intermittent misting and antitranspirant sprays would reduce stresses and in turn improve fruit set of the trees.

### Leaf Water Potential and Stomatal Diffusive Resistance

Midday leaf water potential (LWP), measured at several dates in 1975, was significantly increased following intermittent misting and applications of an antitranspirant (AT) (Table 1). The influence of an early-spring AT spray was still significant on May 16, 42 days after application, when LWP of treated trees was about 30% higher (less negative) than that of the control. Leaf water potential of trees receiving the late-spring AT treatment was approx 60% higher than that of the control trees 2-3 days after the AT application. This effect tended to decrease with time but it was still apparent on June 11. Intermittent misting resulted in consistently high values of LWP which were not statistically different from those of trees receiving the late-spring AP spray.

Table 1. Influence of intermittent misting and antitranspirant (AT) sprays on leaf water potential (- bar) at several dates in 1975.

Treatment	Date							
	May 7 (2-3P) <sup>Z</sup>	May 8 (12N-2P)	May 16 (1-2P)	May 22 (11A-1P)	May 22 (1-3P)	May 26 (1-2P)	May 28 (1-3P)	June 11 (12N-2P)
Control	18.77a <sup>X</sup>	14.09a	11.38a	9.00a	10.58a	8.58a	7.93a	8.12a
Early AT (Apr. 4) <sup>Y</sup>	12.32b	9.58b	8.20b	8.66a	10.15a	7.39b	6.64b	7.97a
Late AT (May 5)	6.63c	5.96c	5.17c	5.37b	7.29b	5.17c	5.07c	6.63b
Misting (Apr. 11)	7.51c	6.90c	5.71c	5.86b	6.59b	5.00c	4.71c	5.69b

<sup>z</sup>Time of measurement.

<sup>y</sup>Date of treatment application.

<sup>x</sup>Mean separation (unlike letters) in columns by Duncan's multiple range test, 5% level.

Leaf stomatal diffusive resistance ( $R_{sto}$ ) was also significantly increased by AT (Table 2). The influence of the early-spring AT spray was negligible, however, on May 7 (35 days later). Leaf stomatal diffusive resistance for the late-spring AT treatment was about 4 times higher than for the control on May 7, 2 days after treatment application. Between May 8 and 15, the  $R_{sto}$  of treated trees dropped considerably. Even so, it remained approx twice as high as the control until June 27.

In the 1977 season, simultaneous and more frequent readings of LWP and  $R_{sto}$  were obtained. Typical daytime cycles of environmental and plant water relation measurements are shown for an early (Fig. 1) and a late (Fig. 2) postbloom date. The LWP and  $R_{sto}$  tended to cycle together, decreasing during the day with min values around 12 noon on the east side of the tree and at about 2 PM on the west side. Minimum LWP values were invariably reached on the west side while lowest readings of  $R_{sto}$  were reached in most instances on the east side. The LWP of untreated trees was inversely related to VPD up to early afternoon (12 N- 2 PM), after which time LWP and  $R_{sto}$  increased while the VPD increased or leveled off. A better relationship was evident, regardless of daytime hr, between LWP and leaf transpirational flux ( $VPD/R_{sto}$ ), a value proposed by Elfving et al. (61). These authors concluded there was a curvilinear inverse relationship between daytime LWP and the ratio  $VPD/R_{sto}$ , under nonlimiting soil moisture supply (tensiometer  $> 0.3$ ), and when  $R_{sto}$  was measured on shaded leaves. Measurements in this experiment were made on exposed leaves and the vapor pressure gradient from leaf to air may not have been reasonably estimated by the air VPD. Also, soil moisture tensions were not known. Even so, a good LWP to flux relationship was obtained (Figs. 1 and 2).



Table 2. Influence of antitranspirant (AT) sprays on leaf stomatal diffusive resistance (sec/cm) at several dates in 1975.

Treatment	Date								
	May 1 (12N-2P) <sup>z</sup>	May 2 (2-3P)	May 7 (11A-1P)	May 8 (2-3P)	May 15 (11A-1P)	May 16 (11A-1P)	May 26 (11A-12N)	May 28 (11A-12N)	June 27 (1-2P)
Control	4.63b <sup>x</sup>	3.55b	3.61b	4.46b	3.94b	6.81b	5.66b	5.29b	5.12b
Early AT (Apr. 4) <sup>y</sup>	5.86a	4.67a	3.82b	5.19b	4.25b	6.60b	6.11b	5.94b	5.62b
Late AT (May 5)	-	-	13.99a	16.40a	9.38a	10.13a	9.14a	8.98a	9.56a

<sup>z</sup>Time of measurement.

<sup>y</sup>Date of treatment application

<sup>x</sup>Mean separation (unlike letters) in columns by Duncan's multiple range test, 5% level.

Figure 1. Typical daytime water relation and environmental measurements on an early postbloom date, May 16, 1977. Influence of an antitranspirant (AT) spray on leaf stomatal diffusive resistance ( $R_{sto}$ ) and of the AT and intermittent misting on leaf water potential (LWP) of east and west sides of trees. Air vapor pressure deficit (VPD) and VPD/ $R_{sto}$  ratio (east and west) data are presented also. Misting started Apr. 23 and the AT was applied Apr. 25.

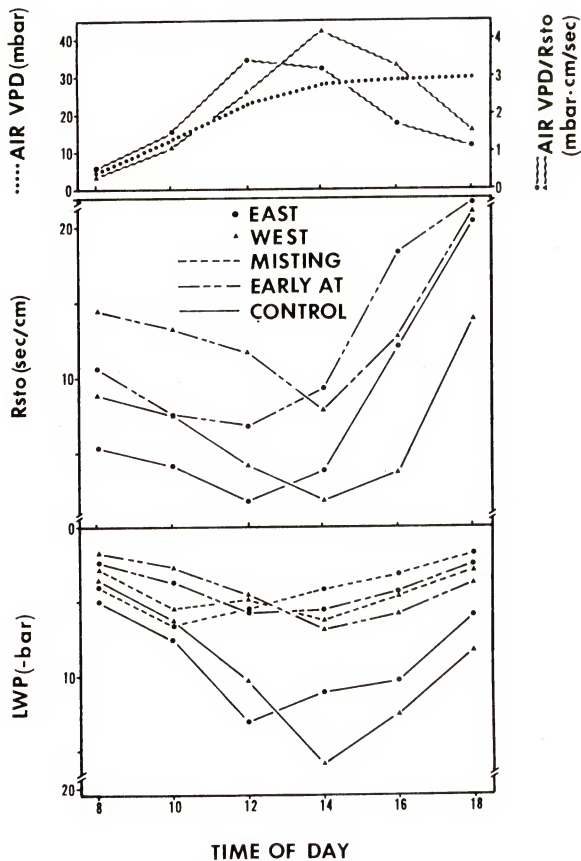
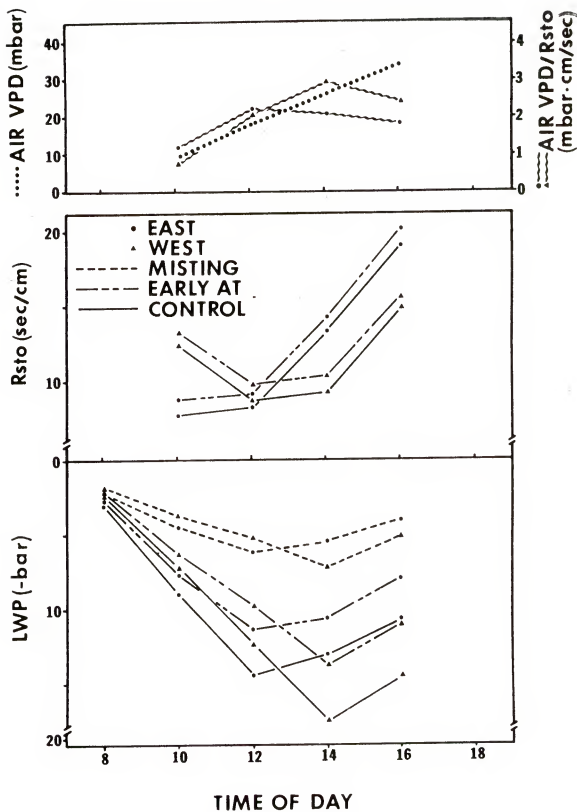
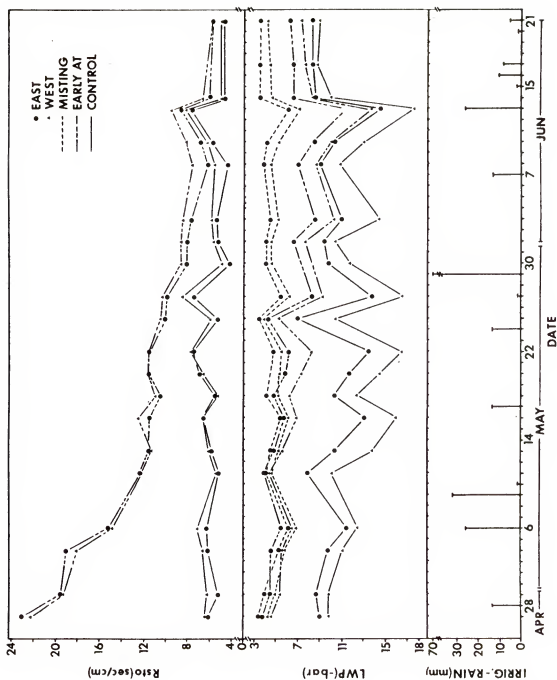


Figure 2. Typical daytime water relation and environmental measurements on a late postbloom date, June 13, 1977. Influence of an antitranspirant (AT) spray on leaf stomatal diffusive resistance ( $R_{sto}$ ) and of the AT and intermittent misting on leaf water potential (LWP) of east and west sides of trees. Air vapor pressure deficit (VPD) and VPD/ $R_{sto}$  ratio (east and west) data are presented also. Misting started Apr. 23 and the AT was applied Apr. 25.



As in 1975, the influence of intermittent misting and AT on LWP, and of AT on  $R_{sto}$  were verified (Figs. 1, 2, and 3), with differences being more conspicuous during midday hr. In the early postbloom period (Fig. 1), the LWP of mist- and AT-tested trees were similar and considerably higher than the control. During this earlier period the overhead sprinklers were usually activated after 10 AM every day when ambient air temp reached values above 27°C. This explains why the LWP values of the misting plot trees were so close to those of the controls up to that time of the day. In the warmer late postbloom period (Fig. 2) the sprinklers were activated shortly after 8 AM and the influence of misting on LWP was pronounced much earlier in the day. Differences in LWP and  $R_{sto}$  between AT-treated trees and the controls were less pronounced in this period. The decrease in the influence of the AT treatment on LWP and  $R_{sto}$  with time is more apparent in Fig. 3 where min daily values of these parameters are shown for all sampling dates during the stress period. On May 16, LWP on the west side of the control trees reached a min value of -16 bars, which was 9 to 10 bars more negative than the min LWP of AT- and mist-treated trees. On June 13, at approx the end of the period of measurements, min LWP of the control trees was -18 bars which was 4 and 11 bars more negative than the min values reached at the AT and misting trees, respectively. Changes in LWP of control and AT trees were accompanied by reciprocal changes in their respective  $R_{sto}$  values. Leaf water potential values of -16 to -18 bars in citrus may reasonably be considered as indication of fairly high levels of water stress. This assumption is based on the work of Elfving et al. (61) which showed that under nonlimiting soil water conditions (tensions < 0.3 bar) orange trees had min LWP of approx

Figure 3. Influence of an antitranspirant (AT) spray on daily minimum leaf stomatal diffusive resistance ( $R_{sto}$ ) and of the AT and intermittent misting on daily minimum leaf water potential (LWP) of east and west sides of trees during the period 4/28 to 6/21, 1977. Irrigation and/or rainfall are also shown. Misting started 4/23 and the AT was applied 4/25.





-12 bars while at limiting soil water (tensions of 0.8 bar) LWP was approx -19 bars.

The data on irrigation and rainfall (Fig. 3) are a rough estimate of the soil water availability. The highest values of LWP were reached immediately following irrigation or rainfall. The LWP dropped during the drying cycle with an accompanying slight increase in Rsto. This is consistent with the studies of Ehlig and Gardner (59) and Gardner and Ehlig (82), which indicated LWP and transpiration decrease simultaneously when the soil dries. The dependence of LWP and Rsto of citrus trees on soil water availability has also been stressed by others (60, 61, 135).

Thus, results from 2 years clearly show the effectiveness of the treatments in improving the water balance of navel orange trees. Intermittent misting increased LWP, the effect remaining relatively unchanged during the entire season. The AT also raised LWP, as a result of increased Rsto. The effect persisted for about 2 months; however, it decreased with time.

Most work on misting irrigation points to this system as an effective means of modifying the plant microenvironment. It has been shown to reduce plant and surrounding air temp, and to increase the relative humidity (22, 29, 85, 177). Those conditions should decrease transpiration losses and reduce plant water stress. There are, however, only a few studies providing direct evidence that plant water balance can be effectively controlled by misting (105, 179). Ryan et al. (179), working with peaches in a planting adjacent to this experiment and at about the same time of the year, reported LWP increases from misting as high as 6 bars. This is less than the 11 bar increments obtained in this work (Fig. 3). The LWP in their experiment, however, was measured

on shaded leaves while unshaded leaves were used in the present work.

The effectiveness of the film-forming AT in increasing  $R_{sto}$  and consequently, raising LWP is in agreement with a no. of studies (17, 43, 46, 48, 49). The decrease in effectiveness of the AT film with time, however, is not entirely consistent with similar research (4, 7). Valid comparisons between the persistence of AT films are difficult. The time they remain effective is quite variable, depending on a no. of factors which include kind and concn of the AT, plant species and stage of growth, and environmental conditions. The steady decrease in the effectiveness of the AT during a 2-month period (Fig. 3) is in disagreement with Albrigo's work (7). He showed Vapor Gard at 3% concn significantly reduced water loss of potted orange trees with only a small change in effectiveness 2 months after application. It should be considered, however, that in the present experiment Vapor Gard was used at 2% concn and sprayed over enlarging leaves of trees in the spring flush of growth. Davies and Kozlowski (50) showed scanning electron micrographs which suggested AT films crack over the stomatal openings, possibly as a result of guard cell movement. Thus, it is reasonable to assume the decrease in effectiveness of the AT with time was due to rupturing of the AT film, which exposed some unprotected leaf surface.

#### Plant Temperature

Limited data on plant temp were obtained in 1975. Midday temp of unexposed leaves were measured during 2 distinct periods in the spring, each starting soon after an AT application.

Average midday leaf temp of the early AT treatment was increased  $0.41^{\circ}\text{C}$  and that for intermittent misting was reduced by  $3.06^{\circ}\text{C}$  in the early period (Table 3). During the late period (Table 4) the late AT spray caused a  $0.65^{\circ}\text{C}$  increase in leaf temp while misting reduced it by  $4.40^{\circ}\text{C}$ . Maximum leaf temp differences were obtained at 2 PM in both periods. The highest leaf temp differences occurred in the late period when ambient air temp were also higher.

In 1977, temp data were obtained from an experiment that differed in several respects from the 1975 work. Daytime plant temp measurements were obtained for canopy air, and sun-exposed leaves and fruits, instead of midday temp of unexposed leaves only. Fruit temp, however, were measured only during the late period that followed the late-spring AT spray.

Solar radiation, ambient air, canopy air, leaf, and fruit temp fluctuated during the day. Maximum values were reached from 1 to 3 PM, except for ambient air temp which tended to peak about 3 PM and to remain relatively unchanged up to 6-7 PM (Figs. 4 to 8). Leaf (Figs. 4 and 5), canopy air (Figs. 6 and 7), and fruit (Fig. 8) temp were always slightly lower than ambient air temp (Figs. 4 to 8) during the early morning and evening hr. They tended to be higher on the east than on the west side of the tree during the morning and early afternoon. The temp of the west side, however, became higher than those of the east side during the remaining daytime hr. Peak temp values on the east were reached 1 to 2 hr earlier than on the west side. Leaf temp tended to be higher than fruit temp, which in turn were higher than canopy air temp during most of the daylight hr, for their respective period of measurements. No differences, however, were apparent between leaf and

Table 3. Influence of intermittent misting and an early-spring antitranspirant (AT) spray on midday leaf temperature for the period 4/21 to 5/6, 1975.<sup>2</sup> Ambient air temperature and solar radiation (cal/cm<sup>2</sup>/min) are also presented.

Time of day	Treatment			Ambient air temp	Solar radiation	$\Delta$ temp (EAT-C)	$\Delta$ temp (M-C)
	Early AT (EAT)	Control (C)	Misting (M)				
12	26.22	26.32	23.88	24.99	1.13	-0.10ns <sup>y</sup>	-2.44**
13	28.01	27.38	24.38	26.61	1.15	0.63**	-3.00**
14	28.94	28.20	24.57	27.47	1.11	0.74**	-3.63**
Avg	27.83	27.37	24.31	26.45	1.13	0.46**	-3.06**

<sup>2</sup>Misting started 4/11 and AT applied 4/4; values are means of 12-15 days.

<sup>y</sup>\*\*Statistically significant at the 1% level, t-test; ns = not significant at the 5% level.

Table 4. Influence of intermittent misting and a late-spring antitranspirant (AT) spray on midday leaf temperature for the period 5/7 to 6/7, 1975.<sup>2</sup> Ambient air temperature and solar radiation (cal/cm<sup>2</sup>/min) are also presented.

Time of day	Treatment			Ambient air temp	Solar radiation	temp (LAT-C)	temp (M-C)
	Late AT (LAT)	Control (C)	Misting (M)				
12	31.69	31.35	27.03	28.86	1.06	0.34 <sup>y</sup>	-4.32 <sup>**</sup>
13	32.35	31.71	27.28	29.52	1.14	0.64 <sup>**</sup>	-4.43 <sup>**</sup>
14	32.50	31.68	27.23	29.96	1.03	0.82 <sup>**</sup>	-4.45 <sup>**</sup>
Avg	32.30	31.59	27.19	29.47	1.08	0.61 <sup>**</sup>	-4.40 <sup>**</sup>

<sup>2</sup>Misting started 4/11 and AT applied 5/5; values are means of 12-15 days.

<sup>y</sup>\*, \*\*Statistically significant at the 5% and 1% levels, respectively, t-test.

Figure 4. Solar radiation; ambient air temperature and leaf temperature of east and west sides of trees as affected by intermittent misting and an early-spring antitranspirant (AT) spray during daytime for the period 5/4 to 5/24, 1977. Misting started 4/23 and the AT was applied 4/25.

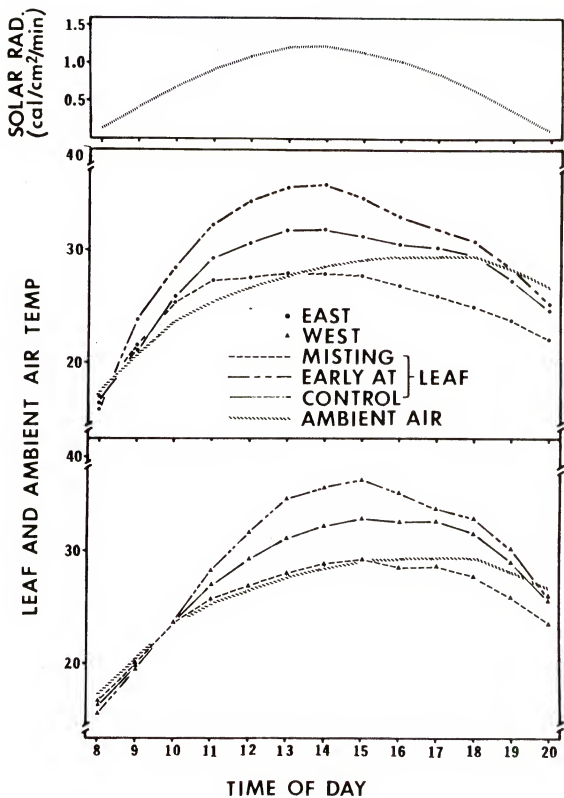


Figure 5. Solar radiation; ambient air temperature and leaf temperature of east and west sides of trees as affected by intermittent misting and a late-spring antitranspirant (AT) spray during daytime for the period 6/1 to 7/6, 1977. Misting started 4/23 and the AT was applied 5/28.



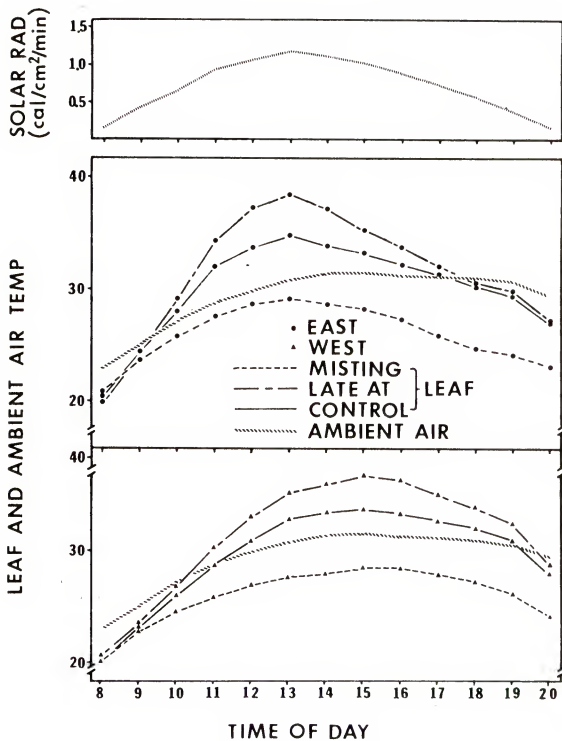


Figure 6. Ambient air temperature and canopy air temperature of east and west sides of trees as affected by intermittent misting and nonmisting treatments during daytime for the period 5/4 to 5/24, 1977. Nonmisting values are pooled data from the control and antitranspirant (AT) plots. Misting started 4/23 and the AT was applied 4/25.

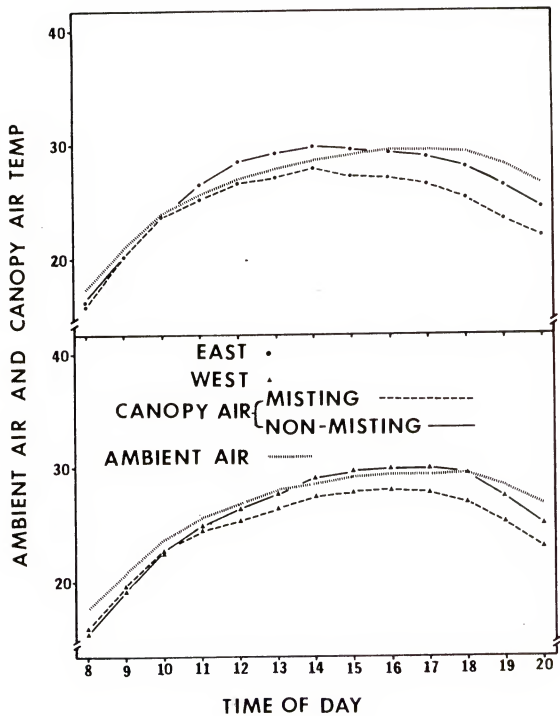


Figure 7. Ambient air temperature and canopy air temperature of east and west sides of trees as affected by intermittent misting and nonmisting treatments during daytime for the period 6/1 to 7/6, 1977. Nonmisting values are pooled data from the control and antitranspirant (AT) plots. Misting started 4/23 and the AT was applied 5/28.

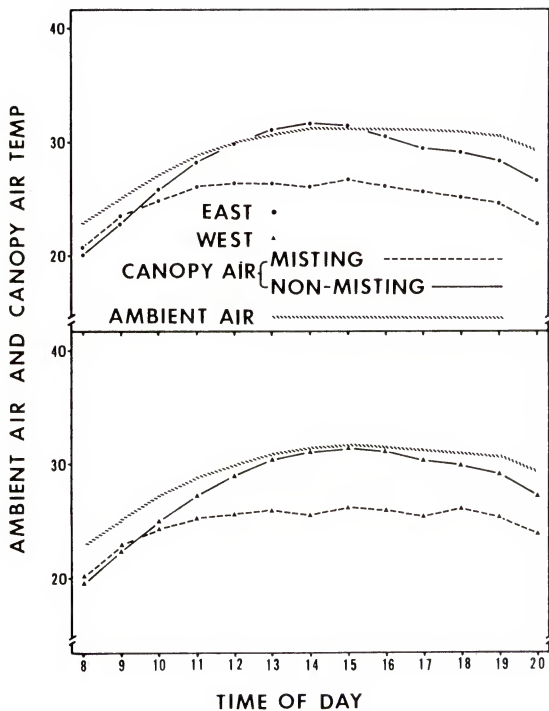
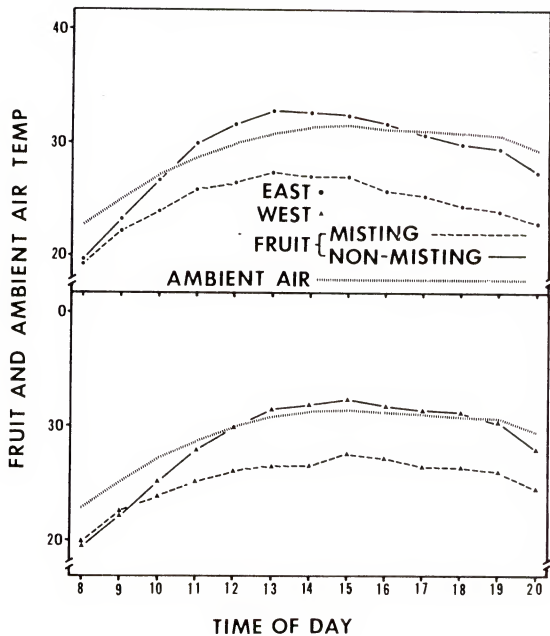


Figure 8. Ambient air temperature and fruit temperature of east and west sides of trees as affected by intermittent misting and nonmisting treatments during daytime for the period 6/1 to 7/6, 1977. Nonmisting values are pooled data from the control and antitranspirant (AT) plots. Misting started 4/23 and the AT was applied 5/28.



fruit temp in the early morning and evening, during the late period. The larger mass and less exposed surface area of the young fruits, somewhat protected by the leaves from the incident radiation, could have accounted for fruits having lower temp than leaves during a large portion of the day. The influence of the treatments on leaf, fruit, and canopy air temp started to be apparent after 10-11 AM with differences being more conspicuous during hr of more intense solar radiation and heat.

Intermittent misting resulted in substantial reductions of leaf (Figs. 4 and 5), canopy air (Figs. 6 and 7), and fruit (Fig. 8) temp. As opposed to other treatments, misting temp values between sunrise and sunset were lower than the ambient air temp outside the mist in most instances. Moreover, temp differences between misting and control treatments tended to remain large and relatively unchanged during the afternoon (1 to 6 PM). This was probably due to the pronounced evaporative demand of the air as a result of the high and relatively unchanged VPD with the higher ambient air temp during those hr. Average afternoon temp differences between misting and control treatments were  $-3.82$  and  $-5.18^{\circ}\text{C}$  for leaves (Figs. 4 and 5), and  $-2.14$  and  $-4.25^{\circ}\text{C}$  for canopy air (Figs. 6 and 7) during the early and late periods, respectively. The average fruit temp differential obtained in the late period was  $-5.22^{\circ}\text{C}$  (Fig. 8). The slightly greater leaf temp differentials obtained in 1977 were probably a result of sampling sun-exposed leaves which were subjected more to the evaporative forces. Also, the modified sequencing cycle of the sprinkling system, which had the working periods extended from 30 sec to 1 min, might have contributed to this difference. In agreement with the 1975 data, leaf and canopy air temp differentials



were more pronounced in the late period during which higher ambient air temp and presumably higher vapor pressure gradients were prevalent. Leaf and fruit temp which differed only slightly prior to misting (control trees) were reduced similarly by this treatment. Air canopy temp, which were lower than leaf and fruit temp prior to misting, were not reduced as much. The higher temp of leaves and fruits prior to misting, as compared to canopy air temp, were due to their greater absorption of radiation. Their greater heat loss following misting was due in large part to the greater evaporative cooling of plant parts as compared to air.

The results presented substantiate several reports (22, 29, 85, 177) that intermittent misting can alleviate plant heat stress by reducing the temp of the plant and its microenvironment through evaporative cooling. It should be noted that in this work misting was provided to 1 or 2 short rows of 5 to 6 trees. More pronounced modification of the microenvironment surrounding the trees might have resulted if misting had been provided to a much larger area.

As in 1975, AT treatments resulted in increased leaf temp. The temp increases, however, were considerably higher in 1977, which was undoubtedly due to the sampling of sun-exposed instead of unexposed leaves. The early-spring AT treatment increased average leaf temp over the control by as much as  $3.38^{\circ}\text{C}$  (Fig. 4). During the late period of measurements, the late AT spray resulted in an average max leaf temp increase of  $3.78^{\circ}\text{C}$  (Fig. 5). No appreciable differences were apparent between the leaf temp increases of the 2 periods. Higher absolute values of leaf temp, however, were observed during the hotter late period. The influence of AT on leaf temp did not decrease with time during either

period. This was evidenced by the nonsignificant regression coefficients obtained when leaf temp increases of each period were averaged for every 3-4 days and linearly related to time. These observations were inconsistent with the previously reported decrease in the influence of AT on  $R_{sto}$ , with time. Contrary to  $R_{sto}$ , however, leaf temp were measured only for relatively short periods of time, 20 days following the early- and 35 days following the late-spring AT sprays, respectively. The decrease in  $R_{sto}$  during those periods may not have been sufficient to result in an appreciable reduction in leaf temp. Furthermore, the thermocouples were frequently changed, being deliberately affixed to selected mature leaves with good degree of AT-film coverage. This could have prevented the manifestation of a declining trend in the AT influence during such short periods of time. The effect of AT in raising leaf temp above the control is presumably a result of reduced cooling from transpiration. Inconsistent results have been reported about the magnitude of the role of transpiration in altering leaf temp (41, 50, 75, 190, 224). Leaf temp is determined by numerous factors and the observed discrepancies could be the result of differences in sampling, leaf properties, and the environment surrounding the leaf, as suggested by Gates (83). Nevertheless, the considerable increases in leaf temp resulting from the AT treatments is in agreement with similar studies. Williamson (224) reported an AT-reduced transpiration of tobacco leaves by 60 to 80% and increased leaf temp 5°C. Slatyer and Bierhuizen (190) reported a 50% transpiration decrease and a 4°C rise in leaf temp of cotton. No effect of AT was observed on canopy air temp during the early-spring or on fruit temp during the period following the late-spring application (Table 5). Simultaneous canopy air temp measurements were

Table 5. Influence of an antitranspirant (AT) spray on canopy air and fruit temperature of east and west sides of trees from 10 AM to 4 PM.

Time of day	Canopy air <sup>z</sup>						Fruit <sup>y</sup>					
	AT		Control (C)		$\Delta \text{ temp}^x$ (AT-C)		AT		Control (C)		$\Delta \text{ temp}^w$ (AT-C)	
	East	West	East	West	East	West	East	West	East	West	East	West
10	24.09	22.43	23.89	22.65	0.20	-0.22	26.44	25.18	26.42	25.04	0.02	0.14
11	26.64	24.85	26.39	24.96	0.25	-0.11	29.77	27.84	29.86	27.79	-0.09	0.05
12	28.21	26.21	28.47	26.42	-0.26	-0.21	31.51	29.83	31.57	29.73	-0.06	0.10
13	29.01	27.58	29.23	27.70	-0.22	-0.12	32.72	31.28	32.75	31.27	-0.03	0.01
14	29.69	28.85	29.79	29.00	-0.10	-0.15	32.54	31.81	32.56	31.71	-0.02	0.10
15	29.45	29.55	29.43	29.62	0.02	-0.07	32.29	32.28	32.29	32.21	0.00	0.07
16	29.07	29.76	29.17	29.81	-0.10	-0.05	31.55	31.93	31.57	31.99	-0.02	-0.06

<sup>z</sup>Values are means of 19-21 observations during the period of 5/4 to 5/24, 1977, which followed the early-spring AT treatment applied on 4/25.

<sup>y</sup>Values are means of 23-27 observations during the period of 6/1 to 7/6, 1977, which followed the late-spring AT treatment applied on 5/28.

<sup>x,w</sup>All temp differences were not significant at the 5% level, t-test.

not consistently taken for the control and AT plots during the late period. It was assumed canopy air temp were the same for the control and AT-treated trees during this period, on the basis of the results obtained in the early period. Thus, the nonmisting values of canopy air and fruit temp shown in Figs. 6 to 8 are pooled data from the control and AT plots. Canopy air temp of nonmisting trees (Figs. 6 and 7) were very close to ambient air temp tending to be slightly higher during peak temp hr. The lack of any AT effect on fruit temp is clearly an indication that the few stomata present in the surface do not allow evaporative cooling to play a meaningful role in the heat dissipation of the fruit.

#### Fruit Set, Size, and Quality

Percent fruit remaining was used as the best estimate of fruit set and yield because the variability in the tree size and amount of bloom precluded the use of yield in kg per tree as a quantitative measurement of treatment response.

Fruit abscission in all trees was most intense during May with very little drop occurring after June 30, when treatment differences were apparent and statistically significant (Fig. 9). Intermittent misting and the early-spring AT spray resulted in increased % fruit remaining in 1975. In contrast, trees receiving a late-spring AT spray, when leaves of the spring flush were fully expanded, had significantly lower % fruit remaining than the controls.

Results of a somewhat modified experiment in 1977 (Fig. 10) show a smaller % fruit remaining difference between intermittent misting and the control (10% as compared to 17% in 1975). Even so, the difference

Figure 9. Influence of intermittent misting, early- and late-spring antitranspirant (AT) sprays on % fruit remaining at monthly intervals, 1975. Misting started Apr. 11 and the AT was applied Apr. 4 and May 5. Approximate full bloom date Mar. 20; end of June drop June 30. Unlike letters differentiate means along the vertical axis.

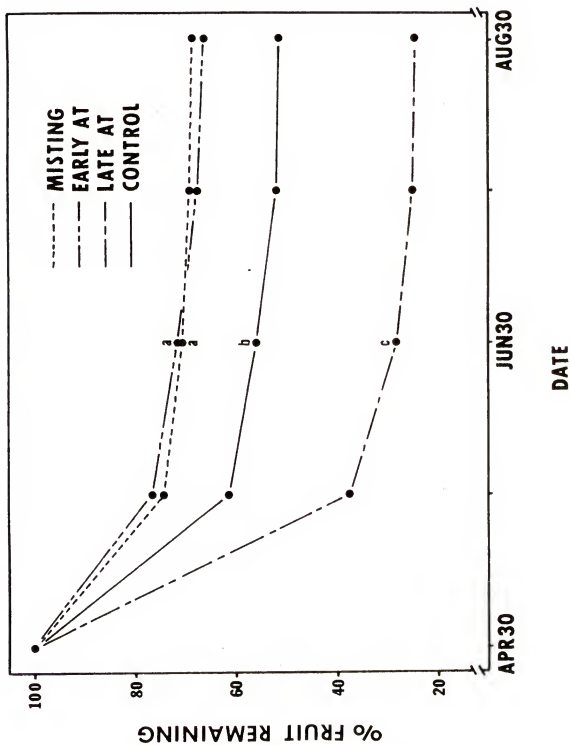
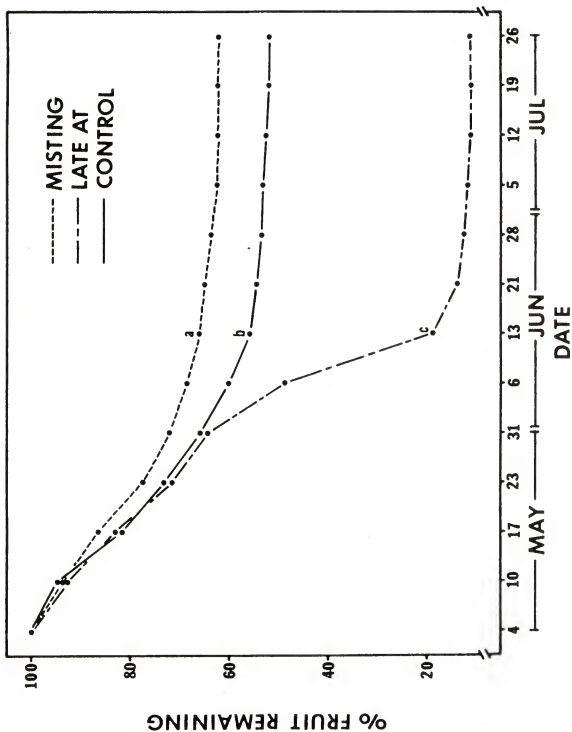


Figure 10. Influence of intermittent misting and a late-spring antitranspirant (AT) spray on % fruit remaining at weekly intervals, 1977. Misting started Apr. 23 and the AT was applied May 28. Approximate full bloom date Mar. 28; end of June drop June 28. Unlike letters differentiate means along the vertical axis.





was statistically significant at the June 13 counting, after which little additional fruit drop occurred. A late-spring AT spray was followed by abnormally heavy leaf and fruit drop confirming results obtained in 1975. The excessive drop of fruits started about 7 days after the AT spray with few fruits remaining on the trees after June 13.

In evaluating the effect of the treatments upon fruit set, the information obtained on plant water balance and temp is relevant. Intermittent misting and the early-spring AT spray resulted in similar increases in % fruit remaining over the controls. Both treatments were also effective in improving the plant water status although the AT influence decreased gradually with time. On the other hand, the AT increased leaf temp and misting reduced it. Thus, the increased fruit set can be directly related to the improved water status during an early spring period following the petal fall whereas the differences in leaf temp were apparently immaterial. In contrast, the late-spring AT resulted in lower % fruit remaining than the control even though the treatment was also effective in reducing plant water stress. The AT treatment increased leaf but not fruit temp which were lower than leaf temp most of the day. This suggests fruit temp was unimportant in the fruit set process. Thus, the excessive fruit drop following the late spring AT spray seems to be related to the increased temp of the leaves which during the hotter, late spring period reached absolute temp values substantially greater than those of AT treatment leaves in the early spring.

It is quite possible that the effects of all treatments on % fruit remaining could have been primarily through changes in the homeostatic hormonal balance of the plant as interpreted by Osborne (163). Critical work on the postulated hormonal balance is, however, scanty. On the

other hand, there is a relatively large body of evidence indicating the level of fruit set in citrus is determined by the amount of photosynthetic products available to the developing fruit (134, 153, 155, 157, 164, 171). The explanation for the effects of treatments on % fruit remaining will be sought on these grounds.

The positive influence of intermittent misting on fruit set in this work is supported by a recent study in California (26) which indicated 15 to 35% increases in fruit set of navel oranges due to misting. The improved water status reported herein should have favored reduced mesophyll resistance to  $\text{CO}_2$  diffusion ( $r'_m$ ) as noted by Gaastra (72) and Kriedemann (135). The average temp of sun-exposed leaves, under misting, from 10 AM to 6 PM, ranged from 24 to 29°C during the season (Figs. 4 and 5). These values are within the optimum range for net photosynthesis of citrus leaves reported by Kriedemann (133). He demonstrated net photosynthesis of citrus leaves remained high and unchanged between 20 to 30°C, with little reduction at 35°C; however, there was a sharp drop from 35 to 40°C. Moreover, it has been reported (105, 136) intermittent misting raised the relative humidity of ambient air surrounding the trees, keeping transpiration at a low level, leaves turgid, and stomata open during hr of intense solar radiation and heat. Even though such measurements were not made in this experiment, it is assumed the conditions created by intermittent misting resulted in higher rates of photosynthesis and thereby favored increased fruit set.

The increased fruit set resulting from the early-spring AT spray has not previously been reported. Increased yields from AT treatments have, however, resulted from increased fruit size (46, 49, 77). Assuming photosynthates as the limiting factor in fruit set, the influence of AT

on water balance, leaf temp, and gas exchange become important. The increased LWP resulting from the AT treatment should presumably have favored lower r'm. The increased LWP was a consequence of augmented Rsto which resulted in increased leaf temp. Average leaf temp of sun-exposed leaves of AT-treated trees, from 10 AM to 6 PM, ranged from 24 to 36°C for a 20-day period following the AT spray (Fig. 4). Leaf temp of control trees ranged from 24 to 33°C during the same period. The leaf temp range of AT-treated trees barely exceeded the limits within which high net photosynthetic rates occur (133). As the leaves expanded during the spring, the AT effectiveness decreased with accompanying reduction in Rsto. This, it is assumed, resulted in less restriction to CO<sub>2</sub> exchange at a time leaves of the spring flush of growth became fully grown and presumably started to function as the source of photosynthate to the developing fruit (134, 171). Thus, the early-spring AT spray apparently resulted in a favorable balance of conditions for net photosynthesis and fruit set.

The reduced fruit set resulting from the late-spring AT spray is consistent with recent work (155) which showed slightly reduced fruit set resulting from applications of a plastic film-forming AT (Acropol). In the present work, the average temp of sun-exposed leaves of AT-treated trees, from 10 AM to 6 PM, ranged from 27 to 39°C for a 35-day period following the late-spring AT spray (Fig. 5). Leaf temp of control trees ranged from 26 to 35°C during the same period. The upper leaf temp limit of the range for AT-treated trees was well within the range where net photosynthesis is supposed to drop sharply (133). The AT sprayed on fully enlarged leaves undoubtedly formed a more continuous film over the surfaces, as indicated by Albrigo (4), and presumably a longer-lasting

coverage. Thus, these factors obviously could have restricted  $\text{CO}_2$  exchange substantially. On the other hand, the excessively high leaf temp could have resulted in reduced carboxylation activity. This, in conjunction with restricted  $\text{CO}_2$  exchange could have caused a drastic reduction of net photosynthesis and consequently led to fruit abscission.

It is a common concept that the alleviation of environmental stresses during the postbloom and fruit-setting periods controls the fruit drop problem of navel oranges. The results of this experiment confirm the influence of the environment on fruit set but the size of the differences was small, suggesting the regulation of the environment alone will not completely overcome the low yields characteristic of navel oranges in many citrus growing regions. The atypical condition of the bloom and trees, however, precludes the development of firm conclusions from these data. More mature trees in better condition might well have responded more favorably. Further trials would be needed to assess the potential yield increases on a quantitative basis.

Fruit size, estimated as wt/fruit in 1975 (Table 6) was increased by all treatments. The largest fruits were obtained with intermittent misting followed in descending order by early- and late-spring AT applications. The differences in size, however, were small among fruits from treatment trees. Differences in fruit size were due to treatments rather than to differences in crop load because data (Table 6) show misted trees had the largest crop load while trees receiving the early-spring AT spray were comparable to the controls.

All treatment fruit had less % TSS in the juice. This was probably a dilution effect due to increased fruit size. Treatments intended to improve plant water status, like intermittent misting and AT sprays,

Table 6. Influence of intermittent misting and antitranspirant (AT) sprays on size (wt/fruit) and total soluble solids (TSS) of the juice, Nov. 1975. Total crop load (yield/tree) is also presented.

Treatment	Wt/fruit (g)	TSS (%)	Yield/tree	
			Fruit wt (kg)	No. fruits
Misting (Apr. 11) <sup>z</sup>	286.74a <sup>y</sup>	10.36c	30.74	107.70
Early AT (Apr. 4)	274.61ab	10.65b	22.08	81.80
Late AT (May 5)	266.83b	10.23c	9.05	33.50
Control	237.92c	11.24a	19.67	84.20

<sup>z</sup>Date of treatment application.

<sup>y</sup>Mean separation (unlike letters) in columns by Duncan's multiple range test, 5% level.

have nearly always resulted in increased fruit size and dilution of soluble solids (17, 46, 48, 77, 85); however, there are some exceptions (26, 179).

### Plant Water Relations and Stomatal Characteristics

On the presumption that the excessive fruit drop of navel oranges is due to water stress, work was conducted to determine whether they have any features that would dispose them to lose more water and suffer greater water stress than such other seedless cultivars as the satsuma mandarin and the 'Valencia' orange.

### Leaf Water Relations

Typical diurnal cycles of LWP and  $R_{sto}$  for the 3 cultivars are shown in Fig. 11. Curves are the result of pooled data obtained on clear sunny days of the late spring as characterized by hygrothermograph charts (Fig. 12) obtained in a standard 1.2 m ht weather shelter located in the experimental area. In agreement with previous data, LWP and  $R_{sto}$  of all cultivars tended to fluctuate simultaneously, with min values being reached on the west side of the trees at about 2 PM (Fig. 11). The LWP differences among cultivars were greater at peak hr on both sides of the trees. Water stress, as estimated by LWP, was most pronounced on satsuma mandarin followed in descending order by navel and 'Valencia' orange trees. The LWP difference between satsuma mandarin and navel orange was, however, smaller than that between navel and 'Valencia' oranges (approx -1 bar as opposed to -3 bars). Water stress was associated with  $R_{sto}$  in that trees showing more negative LWP also had lower  $R_{sto}$ .

Figure 11. Daytime leaf water potential (LWP) and stomatal diffusive resistance ( $R_{sto}$ ) of east and west sides of trees of 3 commercially seedless citrus cultivars. Each point is the mean of at least 12 measurements made during the period 5/13 to 6/1, 1976.

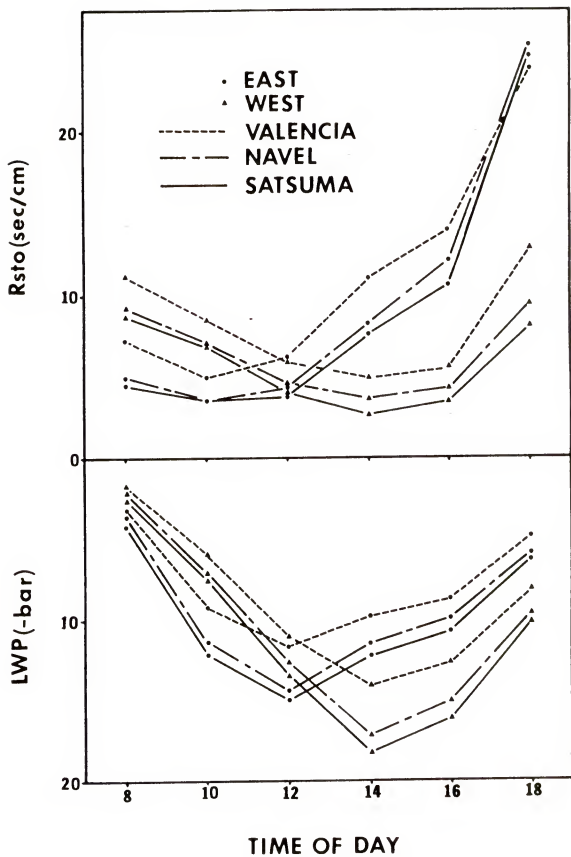
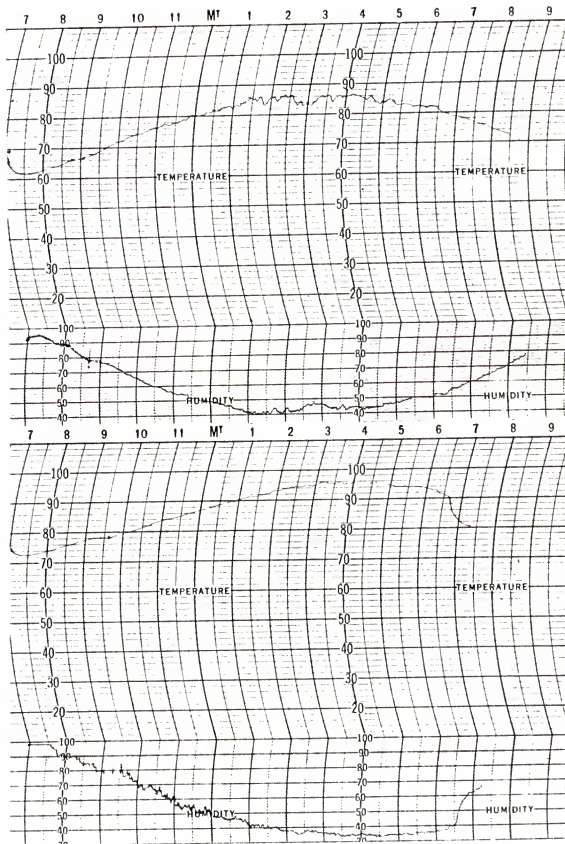




Figure 12. Photograph of hygrothermograph charts showing daytime cycles of temperature ( $^{\circ}\text{F}$ ) and relative humidity (%) prevalent in the experimental area at Eustis, during the period 5/13 to 6/1, 1976.



### Fruit Moisture Losses

Moisture losses of detached fruits were measured on several occasions during the June drop period (late spring and early summer). Typical results are shown for a late date (July 28) in 1976 (Fig. 13) and for an early (May 28) and a late date (July 23) in 1977 (Figs. 14 and 15, respectively). Water losses per unit of surface area of fruit collected late in both years were highest for satsuma mandarin, intermediate for navel, and lowest for 'Valencia' oranges. When fruits were sampled early in 1977, however, rates of water loss were highest for navel orange and lowest for satsuma mandarin. Sufficient no. of samples and measurements were collected to indicate those differences were real. Measurements of fruit water potential at sampling time, not made in this experiment, should substantiate the results obtained. A possible explanation for the conflicting results might be due to the fact the satsuma mandarin bloomed approx 2 weeks later than the orange cultivars. Thus, satsuma fruits were in an earlier stage of development. This difference was more meaningful at the earlier sampling date when changes in fruit development were rapid than at the later sampling date when fruit from all cultivars were rather similar. Thus, the lower rates of moisture loss from satsuma mandarin at the early sampling date could have been the result of either one or a combination of factors. The much smaller mass of satsuma fruit could have resulted in a limited supply of water for transpiration. The total wax/cm<sup>2</sup> on the surface of very young fruit has been shown to be greater than on that of fruit somewhat further developed, after which it again increases (6). Thus, the wax/cm<sup>2</sup> on the satsuma fruit might have been greater than on the slightly older orange fruit at the early sampling date. This would

Figure 13. Cumulative fruit water loss per unit of surface area of 3 commercially seedless citrus cultivars, July 28, 1976. Triangle indicates the time means start to be statistically different (LSD .05).

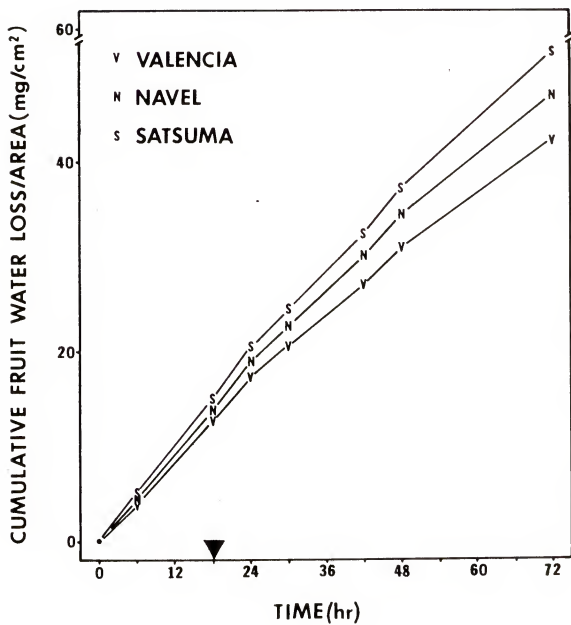


Figure 14. Cumulative fruit water loss per unit of surface area of 3 commercially seedless citrus cultivars, May 28, 1977. Triangle indicates the time means start to be statistically different (LSD .05).

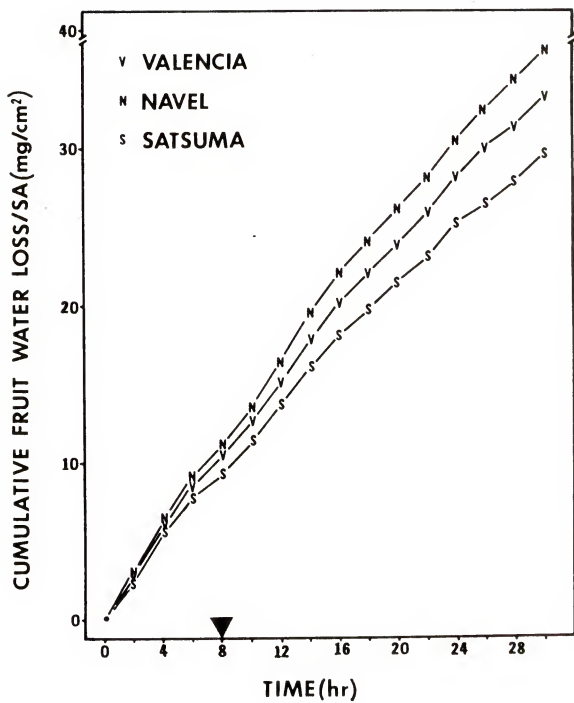
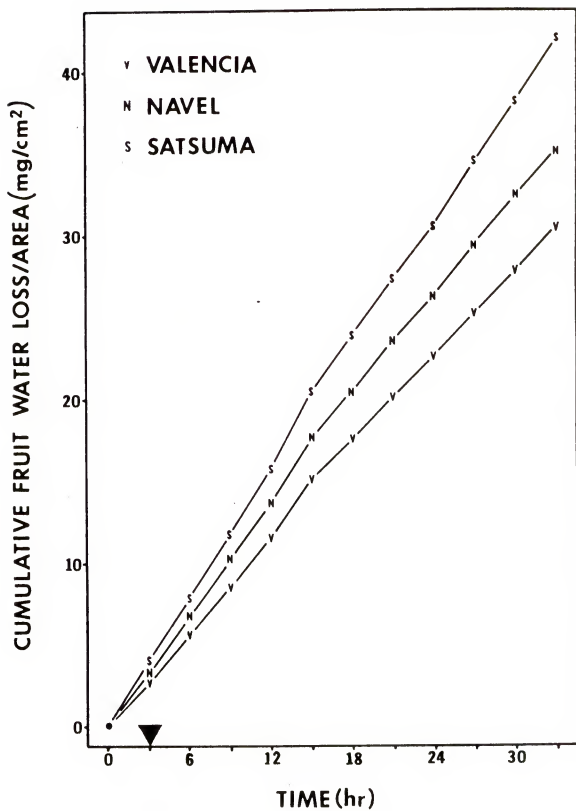


Figure 15. Cumulative fruit water loss per unit of surface area of 3 commercially seedless citrus cultivars, July 23, 1977. Triangle indicates the time means start to be statistically different (LSD .05).





have reduced water loss from the satsuma fruit. Finally, differentiation of stomata on the younger satsuma fruit might not have been completed at the early sampling date (6), thereby reducing water loss. These possible explanations are, of course, speculative.

#### Leaf Stomatal Density

Stomatal densities were determined on fully grown leaves of the most representative size for each cultivar. Satsuma mandarin showed the greatest density of stomata even though leaves were larger (Table 7). Navel and 'Valencia' oranges, which had leaves of similar size, followed in that order. The observed stomatal densities are well fitted to the pattern of water relations of the 3 cultivars. Satsuma mandarin with the highest stomatal density had the lowest  $R_{sto}$  and the most negative LWP. Navel and 'Valencia' orange followed in that order. This sort of relationship is in agreement with a no. of studies with mesomorphic cultivated plants, which indicate rates of water loss were positively related to low  $R_{sto}$  associated with high stomatal density (57, 120, 151, 187, 203).

The few reports on leaf stomatal densities of citrus cultivars show inconsistent results. Bahgat [cited by Hirano (100)] indicated 'Washington' navel as having greater stomatal density than 'Valencia' orange (550 vs. 465 no./mm<sup>2</sup>) which is consistent with the data reported here. On the other hand, Hirano (100) not only ranked the cultivars differently but also reported much lower figures for navel orange and satsuma mandarin (458 and 446 no./mm<sup>2</sup>, respectively). Hirano's data, however, were obtained from trees grown under quite different environmental conditions. Leaves on which Hirano's data are based were larger

Table 7. Stomatal density and area of leaves of 3 commercially seedless citrus cultivars at several sampling dates in 1976.

Sampling date	Stomatal density (no./mm <sup>2</sup> ) <sup>z</sup>			Leaf area (cm <sup>2</sup> ) <sup>y</sup>		
	Satsuma	Navel	Valencia	Satsuma	Navel	Valencia
5/20	627.23	546.25	506.98	34.02	27.58	33.23
5/20	611.93	521.86	502.73	37.31	31.14	27.99
6/2	586.85	524.11	504.30	36.60	32.52	33.71
6/2	608.91	539.96	486.70	35.35	33.63	30.33
7/28	630.16	544.94	498.08	31.69	25.02	29.77
7/29	580.30	533.25	518.28	31.34	28.85	26.89
7/29	558.41	519.94	486.92	34.77	27.35	29.50
8/4	599.47	519.30	499.45	30.72	28.05	30.31
8/4	609.93	518.96	517.73	29.18	29.03	26.23
8/19	618.18	539.07	499.20	31.88	26.68	26.98
Avg	603.14a <sup>x</sup>	530.76b	502.04c	33.29a	28.99b	29.49b

<sup>z</sup>Values are means of 6 leaves, 20 observations per leaf.

<sup>y</sup>Mean area of 6 leaves.

<sup>x</sup>Mean separation (unlike letters) within stomatal density and leaf area by Duncan's multiple range test, 5% level.

than those in this work. Furthermore, the fact that stomatal density data shown here are consistent with water relation measurements suggests they are valid.

### Fruit Stomatal Density

Young, immature fruits sampled late in the spring were used for stomatal countings. The 3 cultivars were ranked identically as for leaf stomatal density but fruit stomatal no. and differences between cultivars were much smaller (Table 8). Variation in fruit stomatal density from sample to sample within each cultivar can be attributed in large part to differences in fruit size. This is evidenced by the significant negative linear correlation coefficient ( $-0.85$ ) obtained between the pooled stomatal density and fruit surface area of the 3 cultivars. Fruit stomatal densities of the respective cultivars were consistently 17 to 18% of the stomatal densities of the leaves. These values are close to the figures reported by Kaufmann (117) and Rokach (178) for young orange fruits.

Rates of fruit water loss reported earlier were directly related to the stomatal densities of the 3 cultivars. There is some inconsistency among the few known reports as to the role of citrus fruit stomata in controlling water loss (5, 90, 178). The data negating the importance of the stomata in this respect, however, were obtained from mature fruits (90) which might have had many of their stomata nonfunctional due to plugging with wax (5, 6, 9). Moreover, the functioning stomata would be at a lower density on fully expanded mature fruit. The relatively greater no. of unplugged and functional stomata of younger,

Table 8. Stomatal density and surface area of fruits of 3 commercially seedless citrus cultivars for several samples collected at 2 dates in 1976.

Sampling date and no.		Stomatal density (no./mm <sup>2</sup> ) <sup>z</sup>			Surface area (cm <sup>2</sup> ) <sup>y</sup>		
		Satsuma	Navel	Valencia	Satsuma	Navel	Valencia
5/28	1	109.89	108.93	110.84	20.42	35.71	33.96
	2	115.72	117.95	117.74	20.47	37.56	31.77
	3	107.87	100.97	85.77	28.58	51.78	44.59
	4	95.24	94.82	93.69	28.20	51.19	47.08
6/4	5	101.65	98.71	74.80	24.43	37.50	40.82
	6	110.03	95.56	86.54	25.07	37.71	42.28
	7	88.03	80.28	75.47	34.43	48.93	51.17
	8	102.67	80.14	66.60	33.25	53.62	52.25
Avg		103.89a <sup>x</sup>	97.17b	88.93c	26.86b	44.25a	42.99a

<sup>z</sup>Values are means of 6 fruits, 20 observations per fruit.

<sup>y</sup>Mean surface area of 6 fruits.

<sup>x</sup>Mean separation (unlike letters) within stomatal density and surface area by Duncan's multiple range test, 5% level.

immature fruits used in this work could have played a meaningful role in moisture loss.

### Stomatal Dimensions

The cuticle covers the outer surface of the guard cells in citrus leaves, forming a continuous ridge which encloses an antechamber outside the central pore between the ventral walls of the guard cells (148, 208). Measurements of the stomatal pore are difficult to obtain by the dimensions of the outer opening and depth of the chamber can readily be measured from photomicrographs. These outer chambers are somewhat protected from the wind and enclose an atmosphere of relatively high water vapor density. With the stomatal pore open the outer chamber becomes continuous with the substomatal cavity. Thus, both the outer opening and the depth of the chamber can conceivably be expected to offer resistance to gas diffusion but their relative importance has not been established in citrus.

The outer opening dimensions of the stomatal antechamber are given for each cultivar (Table 9). Measurements for the large central stomata, around which the regular size stomata were radially arranged, were recorded separately and not used for statistical purposes. As indicated in Table 9 and illustrated in Fig. 16F, the outer openings of these giant stomata can have linear dimensions about twice as large as those of regular size stomata. Satsuma mandarin had the smallest outer openings while no significant difference was found between those of navel and 'Valencia' oranges (Table 9, Fig. 16). The smaller dimensions of the outer openings of satsuma stomata are in agreement with the well documented inverse relationship between stomatal frequency and size of

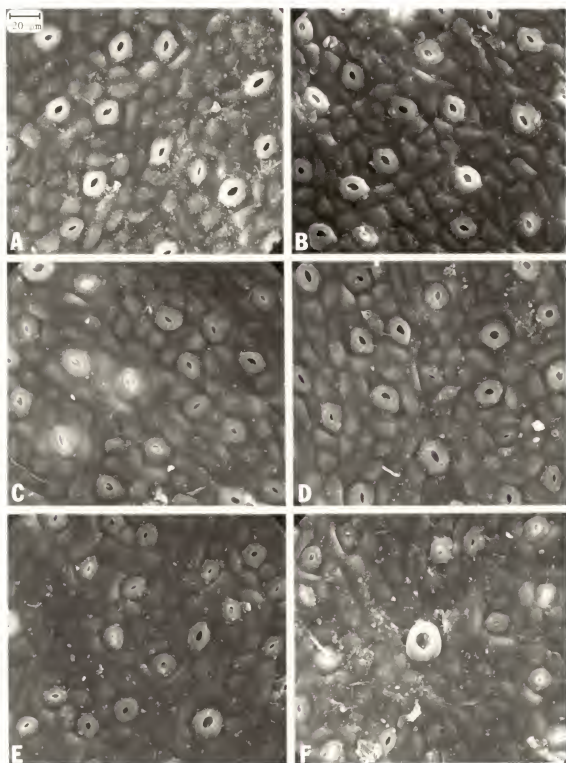
Table 9. Dimensions of outer opening and depth of chamber of leaf stomata of 3 commercially seedless citrus cultivars.

Measurement	Regular size stomata			Large central stomata		
	Navel	Valencia	Satsuma	Navel	Valencia	Satsuma
Outer opening dimensions						
Length ( $\mu\text{m}$ )	5.02a	5.52a	4.55b	10.16	9.41	8.64
Width ( $\mu\text{m}$ )	3.02a	2.92a	2.59b	5.88	5.49	6.41
Ratio L/W	1.94a	1.90a	1.79a	1.53	1.71	1.37
Area ( $\mu\text{m}^2$ )	13.90a	12.77a	9.32b	48.06	40.55	46.33
Depth of chamber ( $\mu\text{m}$ )						
	8.02	8.59	8.99	-	-	-

<sup>z</sup>Mean separation (unlike letters) in rows by Duncan's multiple range test, 5% level. Absence of letters indicates lack of statistical treatment.

Figure 16. Examples of photomicrographs used for measuring outer stomatal opening dimensions on typical lower leaf surfaces of navel orange (A-B), 'Valencia' orange (C-D), and satsuma mandarin (E-F). Relative stomatal sizes are apparent.





the stomata of several species (58, 151, 180, 218). The relations of  $R_{sto}$  and LWP obtained for the 3 cultivars are consistent with the concept that greater stomatal density leads to a lower  $R_{sto}$ . The smaller satsuma stomata presumably reduced the potential for higher  $R_{sto}$  due to more stomata in this cultivar.

The depth of the stomatal outer chamber is illustrated with a stereo pair of photomicrographs shown in Fig. 17 and was measured from pairs of photomicrographs of the kind shown in Fig. 18. Insufficient no. of measurements were made to determine differences between the cultivars (Table 9). The figures obtained, however, were very close to the 7.8  $\mu m$  reported for 'Valencia' orange by Turrell (208).

A review of the data relating to water relations and stomatal characteristics show both LWP and  $R_{sto}$ , which are estimates of water stress, and measurements of water loss from young fruits of the 3 cultivars were related to their respective stomatal densities. Fruit set data were not obtained; however, gross observations indicated clearly there was an excessive amount of fruit drop from the navel trees in comparison with that from 'Valencia' orange and satsuma mandarin. There was no relation, however, between the excessive drop of navel fruits and the estimates of water stress and water loss as might be expected. These results suggest, therefore, the generally accepted assumption that moisture stress is the primary cause of excessive shedding of young navel fruits might not be correct. On the other hand, it is entirely possible that navel oranges have a higher moisture requirement for optimum photosynthate production and flow to the fruit and for the maintenance of a proper hormonal balance than do 'Valencia'

Figure 17. Stereo pair of photomicrographs that demonstrates the dimensions, including depth, of a citrus leaf stomatal antechamber and the antechamber's relationship to the stomatal pore.

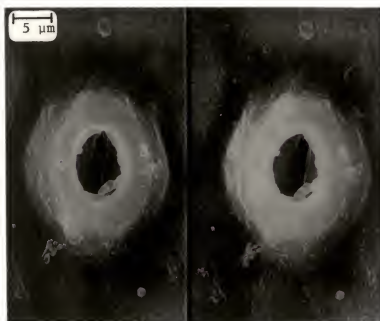
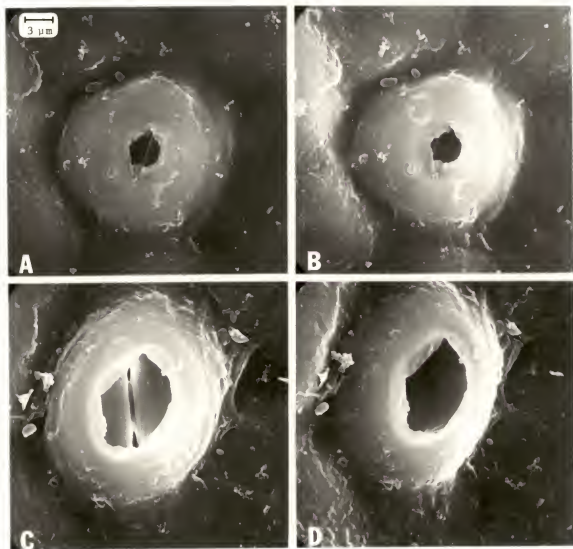


Figure 18. Photomicrographic pairs of leaf stomata (A-B or C-D) demonstrating the technique of measuring the depth of the stomatal antechamber. The distance  $a$  times the  $\tan$  of the change in angle between the photomicrographs of a pair equals the height of an imaginary right triangle or the depth of the chamber.



orange and satsuma mandarin. Work to determine these physiological relations are needed before the complete role of water stress on fruit drop of navel oranges can be determined.

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## BIOGRAPHICAL SKETCH

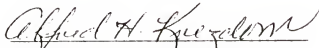
Jose Tarciso Alves Costa was born on August 24, 1942, in Senador Pompeu, Ceará, Brasil. He completed his secondary education at the Ceará State College, Fortaleza, in 1960. He attended the Agriculture College of the Federal University of Ceará for 4 years and received the degree of Engenheiro Agronomo in December 1965. After graduation, he was employed as an Assistant Professor of Horticulture with the Federal University of Ceará, the position he still holds.

In 1970, he entered the University of Arizona, Tucson, and graduated with a Master of Science degree in horticulture, in January 1972. In March 1974, he enrolled in the Graduate School of the University of Florida, where he has been pursuing the degree of Doctor of Philosophy, with a major in horticultural science.

He is married to the former Dagmar Bonfim Medeiros and they have 2 children, Natassia and Erica.

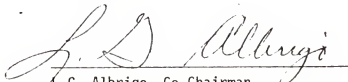


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Alfred H. Krezdorn, Chairman  
Professor of Fruit Crops

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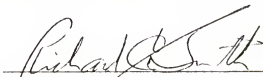
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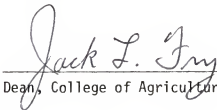


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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 1978



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Dean, College of Agriculture

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